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# Zinc distributions in sediments, the common mussel, Mytilus edulis (L.), the American oyster, Crassostrea virginica (Gmelin), and the commensal pea crab, Pinnotheres ostreum (Say)

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Zinc Distributions in Sediments. the Common Mussel, Mytilus edulis (L.). the American Oyster, Crassostrea virginica (Gmelin). and the Commensal Pea Crab. Pinnotheres ostreum (Say)

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Data Report No. 30

March 17. 1989

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 $\Delta \sim 1$ 

 $\Delta \sim 10^4$ 

 $\bar{A}$ 

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#### **PREFACE**

The data in this report have been obtained in order to estimate parameters in a model of heavy metal bio-accumulation by the American oyster. Crassostrea virginica Gmelin.

Related works:

- (1) Experimental Studies of Zinc-65 Uptake Rates by the American Oyster, Crassostrea virginica with Regard to Salinity, Sediment Concentration. and Body Size. VIMS Data Report No. 29. August. 1988.
- (2) Short Term Uptake Rate of Zinc by the American oyster. Crassostrea virginica. - Relationship Among Body **Size.**  Salinities. and Uptake Rates. In preparation.
- (3) Contribution of Extraneous Materials t<> Variability of Oyster Zinc Bio-concentration Measurements. In preparation.
- **(4) Modelling of Zinc Bio-accumulation in the American**  Oyster. Crassostrea virginica - Influence of Biological and Environmental Factors in Bio-accumulation." Dissertation.

The authors express appreciation for the help of Mr. J. Whitcomb in collecting the oyster and mussel samples.

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#### **DEFINITIOR OF TERMS**

- Soft tissue: organic body of oyster or mussel excluding shell. gut contents. and faecal pellets but including the exoskeleton of the crab.
- Body size: a general term that may mean any one of: shell length, body weight, dry meat weight, or wet meat weight.
- Body weight: dry (meat) weight or wet (meat) weight of soft tissue  $(g$ rams).
- Body burden: the total amount of a metal in soft tissue (micrograms). The metal in gut contents may be included in this report when the amount of the gut content is small.

Concentration: a general term that expresses the mass (of a metal) per unit mass of a material such as water or sediment (dry weight). The unit "ppm" is used interchangably for either microgram/gram of dry material or microgram/ml of solution. Bio-concentration: concentration of metal expressed in mass of the metal

per unit mass (dry weight) of soft tissue (ppm).

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 $\sim 10^{11}$  km  $^{-1}$ 

 $\sim 10^7$ 



 $\sim$   $\sim$ 

 $\sim 10^{-10}$ 

 $\sim 10^7$ 

 $\sim$   $\sim$ 

### **LIST OP ILLUSTRATIONS**

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#### **LIST OF TABLES**

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#### **ABSTRACT**

Oysters and mussels of varying sizes and sediment samples were collected from oyster beds with different salinity regimes of three Virginian coastal plain rivers: Rappahannock River. James River. and Piankatank River.

Zinc concentrations of 1) soft tissues. gut contents. and shells of the oysters. 2) soft tissues of the mussels. 3) pea crabs. and 4) sediment samples were measured with a flame atomic absorption (Flame AA) spectrophotometer. Particulate organic carbon and nitrogen concentrations of the sediments were measured with a carbon-nitrogen analyzer.

The contribution of extraneous materials, such as gut contents, faeces, and pea crabs, to the variability in oyster metal bioconcentration measurements is examined. The effect of salinity differences on bioconcentrations and the relationships between oyster and mussel dry meat weights and body burdens and bio-concentrations also are examined. The relationships are assumed to have the form: uptake equals the product of a constant times weight raised to the power "b" (e.g., a {body size}<sup>b</sup>). Values for the constants a and b are determined for each case.

#### **INTRODUCTION**

It is well known that the American oyster, Crassostrea virginica (Gmelin), accumulates trace metals to concentrations many orders higher than those of surrounding water. Oysters. however, have not been used as biological indicators of metal pollution. at least not as extensively as the common mussel, Mytilus edulis (L.). This may be because there is little information in the literature concerning the relationship between bioconcentration and the various factors that influence the metal uptake rate of oysters.

Assuming first order kinetics. the movement of metals in and out of an organism is

 $\frac{dCo}{dt} = k_1Ce - k_2Co$ 

where Co : concentration of metal in the organism.

Ce : concentration of metal in environment.

 $k_1$  : uptake rate constant.

 $k<sub>2</sub>$  : depuration rate constant.

t time of exposure.

When Ce is constant,

$$
Co = \frac{k_1}{k_2} Ce (1 - e^{-k_2 t})
$$
  
In steady state,  $\frac{dCo}{dt} = 0$   
then,  

$$
\frac{Co}{Ce} = \frac{k_1}{k_2}
$$

When Ce is the total concentration of metal in the water, regardless of the bio-availability, this value,  $\frac{Co}{Ce}$ , is the "Bio-Concentration Factor" (Hamelink 1977).

In the natural environment, it can be asswned that the time of exposure is long enough for the organism to be in steady state in terms of uptake and depuration. As with other metal pollution indicator organisms, it is often assumed that oysters do not regulate metals to any great extent (Phillips 1977). If  $k_1$  and  $k_2$  are constant for all sizes of oysters, then a simple linear regression, i.e., Co=  $\frac{k_1}{k_2}$  Ce + random deviation, would be established for a given set of physiological and environmental conditions.

The total concentration of a metal in the environment and that in the organism, however, are not linearly related (Preston 1966; Boyden 1974, 1977) even though some laboratory uptake and depuration studies suggest that the metal bio-concentration of oysters reaches an equilibrium with ambient concentration (Romeril 1971). The exponential growth rate of the organism and the dilution effect of tissue mass growth makes this body size and body burden per unit mass of tissue relationship complex (Strong and Luoma 1981; Thomson 1982; Simkiss and Mason 1984). Moreover, it has not been understood whether the metal concentration in every cell of the body tissue of oyster changes over the life time (cf. Simkiss and Mason 1984).

The salinity effect, that is, lower trace metal bio-concentrations in higher salinity water and vice versa, has been noticed but the reason for the phenomenon has not been well explained. Information regarding the relationship between body size and metal bio-concentration of an organism provides clues for understanding the bioaccumulation mechanism but the relationship in oysters has not been clearly defined yet.

A major problem in studying the metal accumulation in oysters is that the measured metal body burden of oysters colleeted from the same site at one time shows a wide variation, which makes it difficult to interpret the data. Likely sources of variability are: (1) the use of wet weight instead of dry weight, (2) inclusion of biologically inactive metals associated with sediments in the gut of the organisms, (3) differences in size. Moreover, because of the long biological half lives of trace metals in oyster soft tissues, the metal bio-concentration reflects the cumulative effect of conditions over the life history of the organism rather than just the conditions occurring at the time of collection.

In this study, the relationships between bio-concentrations and the dry meat weights of oysters and mussels were determined. Of interest is whether the relationships change with salinity in each estuarine system. The degree to which the above mentioned extraneous variables contribute to the metal bioconcentration measurements will be examined. The effect of body size on the metal concentration of oysters is hard to evaluate using field samples because metal accumulation is a complex interactive process (cf. Boyden 1974, 1977; Norstrom et al. 1976; Widdows 1978; Strong and Luoma 1981; Phelps et al. 1985; Phillips and Muttarasin 1985); however, it is believed that the analysis of the data from this study will give insights on the bioaccumulation process because 1) oysters with wide range of weights were individually analyzed, 2) oysters were collected from different salinity regimes for each estuarine system making it possible to separate the salinity effects from the body weight effects,  $3)$  many extraneous variabilities were eliminated by excluding gut contents and pea crabs and using dry weights, and 4) seven out of nine samplings were done in a short time span (1 week) eliminating seasonal effects.

Zinc is chosen because it is the metal most accumulated by oysters and by mussels. its bioaccumulation process by mussels has been extensively studied by many authors. its radioactive isotope zinc-65 has a relatively long half life (34.4 weeks) and contamination of the environment by the radioactive material is of concern. and it is a physiologically important element with a long biological half life (300 to 900 days) in oyster soft tissues (Wolfe 1970; Seymour and Nelson 1972. 1973). The ubiquitous use of zinc. moreover. as "the sacrificial anode" for crab pots. monitoring instruments, navigational structures, and boats will increase zinc concentrations in some areas where oysters grow and. thus. might pose some health hazards.

Zinc bio-concentration in oysters and in mussels. moreover. is one of the most simple and easy procedures to monitor because of the high concentrations in soft tissues and because zinc measurements by atomic absorption spectrophotometry are not influenced by interferance of other metals or salts in the samples. By monitoring zinc concentrations, one can detect if there is a pertubation in the environmental trace metal concentrations.

#### **MATERIALS AND METBOD;S**

All of the oysters and mussels, except those from Mulberry Island in the James River. were collected by a dredge (Table 1). The samplings were done concurrently with the annual spring and fall "spat fall survey" by Mr. Whitcomb of VIMS. Oysters from Mulberry Island were collected by oyster

tongs. Sediments that were collected coincidentally with oysters were transferred to bottles by a plastic spoon. Oysters were brushed under running sea water to remove adhering mud. After surface water was removed by blotting with paper towels. they were placed in vinyl freezer bags marked with sampling site and date and kept in a freezer maintained at -12  $C^{\circ}$ .

Oysters and mussels were taken out of the freezer and placed in a refrigerator for 6 to 12 hours until the soft tissues were partially thawed. Oyster shells were opened with a stainless steel oyster shucking knife. Mussel shells were opened with a stainless steel paring knife. Soft tissues were separated from the shells with a stainless steel dissecting knife. The shells were marked and kept for later references. When it was judged that an oyster had enough particulate materials to be of concern, the thinned end of a pipette was inserted into the anal opening of the oyster and gut contents were removed by flushing with deionized water. Gut contents and any sedimentary material (mostly faecal pellets and pseudofaeces) inside of the cavity of the oyster shells were collected in a vial. Oysters were examined to find any female pea crabs and, when one was found, the pea crab was put into a separate vial. Thin (approximately 3 mm) strips of oyster shell were cut along the length.

All of the oyster and mussel soft body tissue samples, the oyster gut content samples, the oyster shells (prepared strips or whole shell when small enough to go into vials), and pea crabs were put into pre-weighed plastic "liquid scintillation counter (LSC) vials" and dried at 105  $\texttt{C}^\texttt{O}$  until there were no weight changes. After determining the dry weights. 2 ml of concentrated nitric acid (HNO<sub>3</sub>) were added to each vial (<u>cf</u>. APHA, Standard Metho4s, 1985).

ZINC STANDARDS: Ten milliliters of "Certified atomic absorption standard zinc reference solution 1000 ppm +  $1\%$ " from Fisher Scientific Co., which was zinc oxide in dilute nitric acid solution  $(1 \text{ m1 = 1 mg Zn})$ , and 150 ml of copcentrated nitric acid were put in a 1000 ml volumetric flask and deionized water was added to make a final volume of 1000 ml. This 10 ppm stock standard was diluted with deionized water to make 0.01, 0.05, 0.1, 0.2. 0.5. LO. 2.0. 4·.o. 5.0. and 10.0 ppm zinc. standards.

QUALITY ASSURANCE MEASUREMENTS: All glass vials were made of borosilicate glass. Pipettes were of TFE. Plastic vials and tubes were of either polypropylene or linear polyethylene with polyethylene caps (cf. Robertson 1965; Struempler 1973; Batley and Gardner. 1977). All non-metal instruments and containers were soaked in 2N HCl and rinsed. with deionized water. Prior to use, they were soaked with 2N HNO<sub>3</sub> and rinsed with distilled-deionized water three times. All metal instruments were rinsed with deionized water before and during the use; moreover, the contact of those instruments with samples was kept to a minimum.

For standards and samples, blanks were made following the same procedures as for the standard or the samples but without the metal or the sample. The measurements of the blanks were subtracted from those of the samples. All acids were Fisher "ACS" grade and had no detectable amount of zinc in them. There was no detectable contamination during sample treatments for the atomic absorption spectrophotometer (see Table 2).

SAMPLE DIGESTION: Each sample was transferred into a 150 ml "fleaker". The vial in which the sample was kept was rinsed with 5 ml of concentrated  $HNO<sub>3</sub>$ three times and the 15 ml of the acid was added to the fleaker. The fleaker

was covered with a watch glass and heated to boiling. Deionized water was added to the 50 ml mark and the £leaker was heated to boiling again. The content of the £leaker was transferred to a 100 ml volumetric cylinder. Fifteen milliliters of deionized water was added to the empty £leaker and the water was poured into the cylinder after rinsing the £leaker. This procedure was repeated three times. Deionized water was added to the cylinder to make the final volume 100 ml. The cylinder was shaken vigorously and then an aliquot was transferred to a volumetric flask to make a final dilution with an estimated concentration of around 0.5 ppm zinc. The final diluents were put into acid-cleaned new LSC vials and centrifuged. These samples were used for the atomic absorption spectrophotometer zinc analyses.

ZINC MEASUREMENTS: Samples, prepared blanks, and 0.01, 0.05, 0.1, 0.2, 0.5, LO. 2.0. 4.0. 5.0. and 10.0 ppm zinc standards were measured by a flame atomic absorption (Flame A.A.) spectrophotometry (Instrumentation Laboratory aa/ ae spectrophotometer model "video 12") (wave length 213.9 nm; flame gases air-acetylene; detection limit O. 005 mg/L; sene:itivity O. 02 mg/L; optimum concentration range 0.05 to 2 mg/L). A standard blank and 0.5 ppm and 1.0 ppm standards were measured. After 10 samples were measured. the blank and the standards were measured again. When there were differences in absorbances of the blank and standards, the instrument was checked until the values were in agreement with previous ones and the 10 samples were measured again. These steps were repeated until all of the samples were measured. The analysis of absorbance values showed that the absorbance increased linearly up to 0.5 ppm concentration but it became non-linear at higher

values (Fig. 1). The absorbance curve became too non-linear to be used as a concentration measurements above 2 ppm.

CALCULATION OF ZINC CONCENTRATIONS: The absorbance values up to 0.208 (0.5 ppm) were converted into ppm values assuming linearity. The absorbance values of standards were fit into a non-linear equation  $Y = a (1 - e^{-b X})$ using "SAS NREG" procedure (SAS Inc., 1985), yielding

Absorbance = 1.671473886 (1 -  $e^{-0.266595963}$  PPM).

The equation

PPM =  $(LOG(1.0-ABSORB/1.671473886)) / (-0.266595963)$ 

was used to convert absorbance values from 0.208 to 0.7 (from 0.5 ppm to approximately 2 ppm) to concentration values.

For each sample. the absorbance value was converted to concentration and then multiplied by the final volume of the sample after dilutions to calculate the total amount of zinc  $\left(\frac{g}{m_1}\right)$  x ml = g-zinc) in the sample. The resulting body burden was divided by the dry weight of the organism to get the zinc bio-concentration ( $\frac{g-zinc}{g-drowedoh}$ ).

SEDIMENT NUTRIENT ANALYSIS: Sediment samples were mixed with deionized water and sieved using a stainless steel frame and cloth sieve (No. 230. 63 micrometer opening) to remove large particles. The samples were homogenized and 20 ml aliquots were dried in LSC vials. Particulate nitrogen and carbon contents were measured with a Carlo-Erba "CN Analyzer."

ANALYSIS OF DATA: The relationships between body size (dry meat weight) and zinc concentration were examined by fitting the logarithmic transformed data into linear equations. If we assume that the body burden (Y) of the

individual is related to body weight (X) as a power function (Boyden 1974; Widdows 1978):



Y', the bio-concentration, or weight specific concentration, is related to body weight as follows:

$$
Y' = \frac{Y}{X} = \frac{aX^{b}}{X} = aX
$$
 (b-1)  
that is, (3)

$$
log Y' = log a + (b-1) log X
$$

The significance of each regression coefficient was tested. The significance of the differences among the regression lines was also tested. The correlations of variables among the pea crab zinc concentration data were examined. All of the statistical analyses were performed using "SPSSX" packages (SPSS Inc., 1986).

All of the datum points in the figures (Figs. 2 to 9) are presented in the tables (Tables 3 to 19). Uniformity of presentation will make comparisons within a species easier to make. All figures of oyster body burdens and bio-concentrations have the same scale; another scale was used for mussels.

#### **RESULTS AND DISCUSSIONS**

Oyster Body Burdens and Factors Affecting Bio-Concnetrations

The dry meat weights, zinc body burdens, and zinc bio-concentrations are presented in Tables 3 to 11 for oysters and Tables 17 to 19 for mussels. The mean values of zinc bio-concentrations of oysters show that in both the James and the Rappahannock· Rivers the organisms which live in higher salinity waters have lower soft tissue zinc concentrations than those in lower salinity regime and vice versa (Table 12). This result agrees with that found in previous similar studies. For the same salinity, the zinc bioconcentrations were greatest in the James River and varied as follows: James>Piankatank>Rappahannock River. There werei no James River mussels; however. the mean values of bioconcentrations of these organisms showed the same salinity effect in the Rappahannock River. For the same salinity, Piankatank mussels had a higher mean bio-concentration than those of Rappahannock River (Table 20).

Some of the differences in mean concentrations, however, are believed to be caused by the weight differences among samples. It has been reported by some investigators that there is no size (i.e. weight) effect on zinc bio-concentrations in oysters (eg. Huggett 1975), but it has been shown in a short term experiment that smaller oysters take up radioactive zinc-65 faster than larger ones in an unit time (Mo and Neilson, in preparation). In the present study. the field data also indicate that there is a size effect on zinc bio-concentration.

The body burdens of zinc in oyster soft tissues observed in this study suggest that the body burden increases throughout the life of the organism. The increases were not linear with the dry weight of the organism (Fig. 2. 4, and 6) and that resulted in bio-concentration increase with dry weight (Fig. 3. 5. and 7).

The rate of increase for each group of oysters was determined by regression analyses assuming that eq. 2 and 4 applied. Once the coefficients a and b were estimated for each data set. the mean behaviour for that group could be plotted (eq. 1 in Figs. 2. 4. 6 and eq. 3 in Figs. 3, 5, 7). The values of a and b determined using eq. 2 and those determined using eq. 4 were nearly identical.

Examination of the coefficient b may provide insights on the bioaccumulation process ( $c$ f. Boyden 1977; Phelps  $c$ t al. 1985; Strong and Luoma 1981; Thompson 1982). Values of b (Table 13) were bigger than 1 for all of the 7 site populations suggesting that a net uptake of the metal is occurring throughout the life of the organism (Williamson 1980; Strong and Luoma 1981). In short term laboratory exposure experiments of zinc-65 by oysters (Mo and Neilson. 1988). it was shown that there is a size dependent difference in uptake rate. Metal uptake per unit biomass by smaller individuals of many species is more rapid than that by larger individuals (Strong and Luoma 1981). It is concluded that 1) the zinc bio-concentration of an oyster keeps increasing during its life time. 2) the rate of the increase is reduced as the oyster grows. 3) in a given time period. the increase of bio-concentration is larger than the dilution effect of the tissue growth in any size oysters (Table 14).

The rate of increase in both body burden and bio-concentration was lower in oysters from a higher salinity regime than in oysters from a lower

salinity regime in the James River (Fig. 2 and 3). This supports the suggestion that the uptake rates of oysters of higher salinity regime decrease more rapidly with size than those of oysters in lower salinity (Mo and Neilson, in preparation). This may contribute to the differences in trace metal concentrations at different salinities (lower concentration in higher salinity and vice versa). The salinity effect on body burdens and bio-concentrations were less obvious in the Rappahannock River oysters. The increases of body burden and bio-concentration with body weight were James>Piankatank>Rappahannock and this would partly contribute to the James>Piankatank>Rappahannock concentration differences at the same salinity regimes.

Additionally. oysters in the Rappahannock River grow faster than those in the James River and oysters in high salinity regime faster than those in lower salinity regime (Haven. personal communications). This would make all of the above discussed differences in concentrations and body burdens more pronounced.

It is suggested that, in addition to the free ion activity differences (higher in lower salinity). uptake rate and growth rate differences in different salinity regimes and different estuarine systems contribute to the salinity effects and to the differences in different systems.

Pea Crabs and Other Factors

A pea crab, the commensal Pinnotheres ostreum Say, had been found in an oyster in the previous experiments of the authors (Mo and Neilson. 1988). Its dry weight (0.059 gram) would have comprised 11 % of the combined dry weight of the oyster and the pea crab (0.4867 gram). The radioactivity of the crab after the exposure (t=4.5 days) was only 0.2050 microcurie/gram

dry-weight while that of the oyster was 2.5615 microcurie/gram dry-weight. If the crab was included as part of the oyster tissue. the radioactivity concentration value would drop by 10%. If the crab is a much better regulator of zinc, this reduction would become much more pronounced as the exposure time increases. In this survey, it was found that the percentage of oysters infested with the pea crabs was highly variable from site to site (Table 22). Zinc concentrations of the crabs were roughly an order of magnitude lower than those of their host oysters and dried weights of the crabs were relatively large (Table 23); thus inclusion of the pea crabs would introduce significant individual and site concentration variability (Table 25). Interestingly. the zinc concentration of an host oyster had no correlation with that of its pea crab and the zinc concentration of a pea crab was primarily dependent on the size (dry weight) of the crab.

. Gut contents and other sedimentary materials such as faeces inside of shell cavities showed a considerable dry weight and zinc concentration (Table  $16$ ). Care should be taken not to include these materials in the samples.

The zinc concentrations in oyster shells **were** extremely small compared to those of oyster soft tissues (Table 15) suggesting that the depuration of zinc through its shell formation is of minor importance.

#### Mussels

Zinc body burdens of mussels from the low salinity region of the Rappahannock River were almost linear with the dry weights of the organisms (Fig. 8), i.e., the value of b is about 1 and the metal concentration per

unit body weight is independent of body size (Fig. 9). This suggests that equilibration of concentrations of the metal occurs in the tissues of the organism (Bryan 1976; Williamson 1980; Strong and Luoma 1981). Zinc body burdens of mussels in high salinity Rappahannock River showed the value b was smaller than 1 (Fig. 8). which means (b-1) is negative (Table 21). indicating that bio-concentration decreases with size (Fig. 9).

Table 1. Oyster sampling sites and dates

# **The James River oyster beds:**



**The Rappahannock River oyster beds:** 

...



#### **The Piankatank River oyster bed:**



- \*1: The code represents "(river)site.salinity(.season of collection)". In tables and illustrations. the parts of the code in parentheses are omitted except where the omission may cause a confusion.
- \*2: distance from the river mouth in km
- \*3: approximate annual average salinity
- \*4: month/day/year



 $\sim 10$ 

 $\sim 10$ 

 $\ddot{\phantom{a}}$ 



\*l: actual concentration

 $\sim 10^7$ 

 $\sim$   $\sim$ 

- \*2: measured concentration of diluted sample
- \*3: measured concentration of undiluted sample

 $\ddot{\phantom{a}}$ 

Table 3. Zinc in oysters from Wreck Shoal-James River (Spring-6/15/88)  $\mathcal{L}^{\text{max}}_{\text{max}}$  and  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\bar{z}$ 

 $\sim 10$ 

 $\sim 10$ 



 $\hat{\mathcal{A}}$ 

 $\sim$   $\sim$ 

 $\mathcal{L}$ 

 $\sim 10$ 

Table 4. Zinc in oysters from Wreck Shoal-James River (Fall-10/7 /87)

 $\sim 10^{-10}$ 

 $\sim$   $\sim$ 

 $\sim 10^{11}$ 

 $\ddot{\phantom{0}}$ 



 $\sim 100$ 

 $\sim 10^{11}$ 

 $\mathcal{L}(\mathcal{A})$  and  $\mathcal{L}(\mathcal{A})$ 

 $\sim 10$ 



 $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$  and  $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\sim$ 

Table 5. Zinc in oysters from Mulberry Island-James River (Winter-1/19/88)

 $\sim$ 

 $\sim 10^7$ 



 $\ddot{\phantom{0}}$ 



19

 $\bar{\phantom{a}}$ 

 $\bar{z}$ 

 $\hat{\boldsymbol{\epsilon}}$ 

 $\ddot{\phantom{a}}$ 

 $\sim$   $\sim$ 

# Table 7. Zinc in oysters from Horse Head Rock-James River (Fall-10/7 /87)

 $\mathcal{L}^{\text{max}}_{\text{max}}$  , where  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\frac{1}{2} \left( \frac{1}{2} \right)$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$  ,  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\Delta \sim 10^{11}$ 





 $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$  and  $\mathcal{L}^{\text{max}}_{\text{max}}$ 



 $\label{eq:2} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\,d\mu\int_{\mathbb{R}^3} \frac$ 

 $\label{eq:2.1} \frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\int_{0}^{\infty}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\pi}}\frac{1}{\sqrt{2\$ 

# Table 9. Zinc in oysters of Parrot Rock-Rappahannock River (Fall-10/9/87)



### Table 10. Zinc in oysters from Broad Creek-Rappahannock River (Fall-10/9/87)

 $\mathcal{A}$ 

 $\sim$ 

 $\bar{z}$ 



 $\mathcal{L}_{\mathcal{A}}$ 

 $\hat{\xi}^{(A)}$ 

 $\mathcal{L}^{(1)}$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\bar{t}$ 

 $\sim 10^{-1}$ 

 $\hat{\mathcal{A}}$ 

# Table 11. Zinc in oysters from Ginney Point-Piankatank River (Fall-10/12/87)

 $\label{eq:2.1} \frac{1}{\sqrt{2}}\int_{\mathbb{R}^3} \frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2\frac{1}{\sqrt{2}}\frac{1}{\sqrt{2}}\left(\frac{1}{\sqrt{2}}\right)^2.$ 



 $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$  , where  $\mathcal{L}^{\mathcal{L}}(\mathcal{L}^{\mathcal{L}})$ 

 $\mathcal{L}$ 

 $\mathcal{L}_{\text{max}}$  and  $\mathcal{L}_{\text{max}}$ 

 $\ddot{\phantom{0}}$ 

 $\ddot{\phantom{a}}$ 

 $\hat{\mathcal{A}}$ 

24

 $\hat{\mathcal{A}}$ 

### Table 12. Summary statistics of oyster data

 $\mathcal{L}^{\text{max}}_{\text{max}}$  and  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

### The James River

 $\sim 10$ 

 $\sim 10$ 

 $\sim 10$ 





 $\sim 10^{-1}$ 

SAMPLE ID: C JAMES.MI.14.WINTER n: 18



SAMPLE ID: E JAMES.HH.10.FALL n: 21

 $\Delta$ 

 $\sim 10^7$ 



Table 12 (continued). Summary statistics of oyster data

SAMPLE ID: D JAMES.NR.20.FALL n: 16 Variable Mean Std.Dev. Min:imum Maximum Dry weight (g) 0.588 0.414 0.0263 1.2807 Body burden (1836 2096 1835 88 5962 Bio-Conc. (ppm) 3355 1501 2007 7680

#### The Rappahannock River

 $\mathcal{L}_{\mathcal{A}}$ 



SAMPLE ID: H RAPP.PA.15.FALL n: 17



SAMPLE ID: K RAPP.BC.18.FALL n: 17


Table 12 (continued). Summary statistics of oyster data

 $\hat{\mathcal{L}}$ 

## The Piankatank River



 $\mathcal{L}_{\mathcal{L}}$ 

 $\sim 10^{-10}$ 

 $\sim 10$ 

Table 13. Results of regression analyses of oyster zinc body burden on body weight. Dependent variable is  $log_{10}$  (Body Burden) and independent variable is  $\log_{10}$ (Dry Meat Weight). 'a' is a constant and 'b' is the coefficient of the independent variable (i.e.,  $Y = a + bX$ ).

## The James River



Table 13 (continued).

SAMPLE ID: C JAMES.MI.14.WINTER Multiple R .95666 -------- Analysis of Variance -------R Square **91521** DF Sum of Squares Mean Square Adjusted R Square .90991 Regression 1 5.47862 \_5 .47862 Standard Error .17811 Residual 16 .50759 .03172  $F = 172.69507$ Signif  $F = 00000$ --------- Variables in the Equation --Variable B SE B **Beta**  T Sig T b 1.123115 .085464 .956665 13 .141 .0000 3.810384 .064792 a 58.809 .0000 SAMPLE ID: E JAMES.HH.10.FALL Multiple R .92455 -------- Analysis of Variance -------R Square .85479 .85479 DF Sum of Squares Mean Square Adjusted R Square .84715 Regression 1 1.59148 1.59148 Standard Error .11929 Residual 19 .27036 .01423  $F = 111.84492$  Signif  $F = .0000$ -------- Variables in the Equation -------Variable B SE B Beta T Sig T b 1.304121 .123313 .924548 10.576 .0000 a 4.008342 .039455 101.594 .0000 SAMPLE ID: D JAMES.NR.20 Multiple R .95519 -------- Analysis of Variance -------.91239 DF Sum of Squares Mean Square R Square Adjusted R Square .90565 Regression  $\mathbf{1}$ 3.63351 3.63351  $13$ Standard Error .16383 Residual .34892 .02684  $F = 135.37719$ Signif  $F = 00000$ -~------ Variables in the Equation ------- Variable B SE B Beta T Sig T .955189 11.635 .0000 b 1.059964 .091100 .055946 62 .897 .0000 a 3 .518876

Table 13 (continued).

## The Rappahannock River

 $\sim 10$ 



30

 $\mathcal{L}_{\rm{max}}$  ,  $\mathcal{L}_{\rm{max}}$ 

 $\bar{\mathcal{A}}$ 

Table 13 (continued).

SAMPLE ID: K RAPP.BC.18.FALL Multiple R R Square Adjusted R Square Standard Error .94513 .8.9328 .88616 .15858 ------- Analysis of Variance -------DF Sum of Squares Mean Square Regression Residual 1 15  $F = 125.55339$ 3.15741 .37722 Signif  $F = 00000$ 3.15741 .02515 -------- Variables in the Equation ------- Variable B SE B Beta T Sig T b 1.176272 .104977 .945134 11.205 .0000 a 3.251504 .075988 42.789 .0000

## The Piankatank River

 $\omega_{\rm{max}}$ 



 $\ddot{\Sigma}$ 

Table 14. Results of regression analyses of oyster zinc bio-concentration on body weight. Dependent variable is  $\log_{10}(\text{Bio-Concentration})$  and independent variable b is  $log_{10}(Dry$  Meat Weight). 'a' is a constant and 'b' is the coefficient of the independent variable (i.e.,  $Y = a$ + bX).

## The James River





Table 14 (continued).

SAMPLE ID: C JAMES.MI.15.FALL Multiple R .33893 -------- Analysis of Variance -------R Square .11488 DF Sum of Squares Mean Square Adjusted R Square .05956 Regression 1 .06586 .06586 Standard Error .17808 Residual 16 .50743 .03171  $F = 2.07658$  Signif F = .1689 -------- Variables in the Equation ------- Variable B SE B Beta T Sig T b .123137 .085451 .338935 1.441 .1689 a 3.810400 .064782 58.819 .0000 SAMPLE ID: E JAMES.HH.10 Multiple R .49246 -------- Analysis of Variance -------R Square **.24252 DF** Sum of Squares Mean Square Adjusted R Square .20265 Regression 1 .08656 Standard Error .11929 Residual 19 .27037  $F = 6.08316$  Signif  $F = 0.0233$ -------- Variables in the Equation ------- Variable B SE B Beta T Sig T b .304147 .123316 .492463 2.466 .0233 a 4.008350 .039455 101.592 .0000 SAMPLE ID: D JAMES.NR.20 Multiple R .17958 -------- Analysis of Variance -------R Square .03225 Adjusted R Square -.04219 Standard Error .16383 Regression 1 Residual . DF 13 Sum of Squares Mean Square .01163 .34893 .08656 .01423 .01163 .02684  $F =$  .43320 Signif F = .5219 -------- Variables in the Equation -------Variable b a B .059962 3.518894 SE B  $.091102$ .055947 Beta .179579 T Sig T .658 .5219 62.896 .0000

Table 14 (continued).

# The Rappahannock River



 $\sim 10^{-10}$ 

 $\bar{\beta}$ 

 $\bar{\beta}$ 

Table 14. (continued).



 $\sim 10^{-11}$ 

## The Piankatank River



 $\sim 10^7$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\label{eq:2.1} \begin{split} \mathcal{L}_{\text{max}}(\mathbf{r}) & = \frac{1}{2} \sum_{i=1}^{N} \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \\ & = \frac{1}{2} \sum_{i=1}^{N} \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf{r}) \mathcal{L}_{\text{max}}(\mathbf$ 

Table 15. Zinc in oyster shells



 $\sim 10$ 

\*: Concentration (or total zinc) in oyster soft tissue devided by concentration (or total zinc) in oyster shell.

 $\mathcal{L}_{\mathcal{A}}$ 

-: Missing values

 $\mathcal{L}$ 

 $\frac{1}{2}$ 

 $\sim$   $\sim$ 

 $\sim 10^{11}$  m  $^{-1}$ 

 $\mathcal{L}_{\mathrm{max}}$ 

 $\sim$ 

Table 16. Contribution of gut contents<sup>\*1</sup> to measurements of dry weights and zinc concentrations



\*l: "gut contents" include all of the sedimenteous materials inside of the shell cavity of an oyster such as gut contents, faeces and psuedofaeces \*2: all measurements of oyster tissue are without gut contents \*3: concentration of zinc including dry weights and zinc of both oyster tissue

 $\sim$ 

and gut contents

 $\sim$ 



 $\bar{z}$ 

 $\ddot{\phantom{0}}$ 

 $\ddot{\phantom{0}}$ 

 $\epsilon$ 



 $\bar{\beta}$ 

 $\sim$ 

# Table 18. Zinc in mussels from Parrot Rock-Rappahannock River (Fall-10/9/87)

 $\sim 10$ 

 $\ddot{\phantom{a}}$ 

 $\mathcal{L}$ 



 $\mathcal{L}_{\text{max}}$ 

 $\mathbb{R}^{\frac{1}{2}}$ 

 $\bar{z}$ 

 $\bar{\mathbf{r}}$ 

 $\bar{\mathcal{A}}$ 

 $\hat{\boldsymbol{\epsilon}}$ 

Table 19. Zinc in mussels from Ginney Point-Piankatank River (Fall-10/12/87)

J.



 $\mathbb{R}^2$ 

 $\bar{\mathcal{A}}$ 

 $\tilde{A}$ 

 $\mathcal{A}^{(1)}$  .

Table 20. Summary statistics of mussel data

 $\epsilon$ 

 $\overline{\phantom{a}}$ 

 $\hat{\mathcal{A}}$ 

SAMPLE CODE NUMBER DRY WGT. BODY BURDEN BIO-CONC. CORR.COEF<sup>\*1</sup> (samples) (gram) $\pm$ std.dev.  $\mathcal{W}(g)\pm$ std.dev.(ppm) $\pm$ s.d.



\*1: correlation between mussel dry weights and zinc concentrations. xx denotes that the numbers are significant at 1% level.

Table 21. Regression of mussel zinc body burden on body weight. Dependent variable is  $\log_{10}$  (Body Burden) and indenpendent variable is  $log_{10}$ (Dry Meat Weight). 'a' is a constant and 'b' is the coefficient of the independent variable (i.e.,  $Y = a + bX$ ).



\*l: xx denotes t value being significant at 1% level.

## Table 22. Presence of pea crabs in oysters

 $\ddot{\phantom{a}}$ 



\*l: Number of oysters examined.

 $\langle \cdot \rangle$ 

\*2: Number of oysters with female pea crabs inside of shell.

 $\bar{z}$ 

 $\mathbf{v}_i$ 

## Table 23. Zinc in Pea Crabs

 $\sim 10$ 



 $\sim 10^{11}$  km s  $^{-1}$ 

 $\sim$ 

 $\mathcal{L}(\mathcal{L}^{\mathcal{L}})$  and  $\mathcal{L}^{\mathcal{L}}$  and  $\mathcal{L}^{\mathcal{L}}$ 

\*1: identification number of host oyster

 $\label{eq:2.1} \begin{split} \mathbf{r} &= \mathbf{r} \cdot \mathbf{r} + \mathbf{r} \cdot \mathbf{r} + \mathbf{r} \cdot \mathbf{r} \\ \mathbf{r} &= \mathbf{r} \cdot \mathbf{r} + \mathbf{r} \cdot \mathbf{r} + \mathbf{r} \cdot \mathbf{r} \end{split}$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$  ,  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$  , where  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

 $\sim 10$ 

# Table 24. Contribution of pea crabs to measurements of dry weights and zinc concentrations



\*l: (dry weight of pea crab)/(dry weight of pea crab and oyster) \*2: (body burden of pea crab and oyster)/ (dry weight of pea crab and oyster)

 $\sim 10^{-11}$ 

 $\sim 400$ 

 $\mathcal{L}^{\text{max}}_{\text{max}}$  and  $\mathcal{L}^{\text{max}}_{\text{max}}$ 

Table 25. Zinc and organic contents in bottom sediments

 $\mathcal{A}$ 

Sampling site Zinc Cone. Organic Carbon Organic Nitrogen (ppm-dry wgt.)

The James River:



The Rappahannock River:



The Piankatank River:

 $\bar{\alpha}$ 

 $\mathcal{L}_{\mathcal{A}}$ 

 $\lambda$ 

 $\hat{\gamma}_{\alpha}$ 

Ginney Point

# Absorbance by PPM





























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