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DAMOS

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New London Disposal Site
Monitoring Projects
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Introduction

Natural weathering, runoff, aerial deposition, open water dredged spoil disposal as well as industrial and municipal discharges constitute the major input of trace metals in coastal waters. In Long Island Sound, according to Fitzgerald (1974), more than 80% of the Cu and Zn input appears to be of anthropogenic origin; he further states that "about 90% of the Cu and Zn entering the Sound annually is removed either biologically or geochemically to the sediment". The role of marine bivalve molluscs in extracting trace metals from their environment and concentrating the metals in their tissues is well known. It is one of the biological mechanisms by which trace metals are removed from the aquatic environment. A number of investigators have taken advantage of this unique characteristic of the molluscs, and successfully used them as sentinel organisms in monitoring environmental qualities (Pringle et al., 1968; Shuster and Pringle, 1968; Romeril, 1974; Leatherland and Burton, 1974; Goldberg et al., 1978). Feng and Ruddy (1975) reported that the concentrations of certain trace metals in oysters deployed along the Connecticut coast were associated with the degree of industrialization of the region, while Frazier (1976) observed a relative enrichment of metals in oysters, which reflected patterns of metal contamination in sediment. Comparative studies of trace metals in bivalve molluscs and other marine organisms from within and around disposal sites in the New York Bight have been reported by Greig and Jones (1976) and Greig et al. (1977). In recent years, Phelps and Galloway (1980) have used the blue mussel, *Mytilus edulis*, to assess the condition of Narragansett Bay in Rhode Island. These monitoring systems are important from a food chain point of view, since high concentrations of trace metals in edible bivalve molluscs constitute a health hazard to consumers. Furthermore, the presence of excess amounts of trace metals from anthropogenic activities may impose stresses on marine organisms (Bryan, 1971). For example, cadmium and copper in particular have been shown to be toxic to the growth and reproduction of bivalve molluscs (Shuster and Pringle, 1968; Calabrese et al., 1977).

The uptake of trace metals by bivalve molluscs is dependent on the availability of metals in the environment as well as water temperature, physiological state and the size of the organism (Romeril, 1974). However, the uptake is by no means irreversible; as shown by Feng and Ruddy (1975), oysters from a highly polluted area, when transplanted to an environment of low metal concentrations, show depuration of metals.

From July 1977 through July 1979 more than 1.6 million cubic yards of spoil were removed from the Thames River and disposed in a one square mile area known as the Eastern Long Island Sound Disposal Site which is located ca. two miles south of the river mouth. [Because Resuspension of fine sediment and concomitant release of interstitial water during maintenance dredging of channels and harbors, and subsequent open water disposal of dredge spoils could increase certain nutrients, trace metals and organics in the environment (Nisbet and Sarofin, 1972).] We are, therefore, concerned with the transport and fate of these spoil-associated contaminants in the environment as well as their potential effects on living marine resources, such as the blue mussel. In this investigation we are seeking answers to the following questions:

1. Are there significant increases of trace metals in M. edulis, attributable to open water disposal of dredged spoils or other environmental factors?

2. Are there overt physiological changes in M. edulis, that can be ascribed to the increase in tissue trace metals?

This report analyzes the results of temporal and spatial variations of trace metal levels and the physiological condition expressed as changes in the ratio of wet and dry tissue weight of M. edulis deployed in the vicinity of Eastern Long Island Sound disposal site and reference site. Histological studies which attempt to discern differences, if any, in the development of female reproductive tissues between the reference and experimental populations, are presented in a separate report. Findings on PCB levels in mussels have been reported elsewhere (Arimoto and Feng, 1982).

Experimental

Field Procedures

The blue mussel, Mytilus edulis, was used to establish four monitoring stations (Fig. 1). It is chosen for its ubiquity, sessile existence as well as its recognized commercial and ecological importance; moreover it can function at a wide temperature and salinity range. In addition, it is the principal organism used in the Global Mussel Watch Program under the auspice of the United Nations. A single population from North Dumpling, New York, in Fishers Island Sound was employed as experimental animals in order to minimize variations due to genetic diversity which may exist in local mussel populations. Groups of 24 mussels were placed in polypropylene mesh bags and attached to a PVC platform (1 m x 1 m x 1 m) with concrete footings (Fig. 2). The platform was lowered to the sea bottom by a shipboard winch (Fig. 3) and its position was recorded by Loran coordinates. The platform array consists of a renewable subsurface sonic pinger (Model Ph-10, Johnson Laboratories, Southold, N.Y.) which facilitates relocation of subsequent sampling of mussels, a subsurface pop-up float and a detachable surface buoy which releases the pop-up float when dragged by a surface vessel. Depending on the season and the intensity of dredging activities, mussels were retrieved monthly or bi-monthly from the four stations: D1, D2, D3 and ND by divers using SCUBA.

Ten replicate baseline samples were first collected from North Dumpling, the reference site, for trace metal analysis and wet-dry weight ratio determination when the stations were first established. Sufficient bags of mussels were stocked initially at each station to allow bi-monthly sampling for two years. But restocking was necessary due to increased sampling effort, predation by blackfish, mortality and the irretrievable loss of the platform. Whenever a station was restocked or reestablished, replicate baseline samples were always obtained.

Laboratory Procedures

During each sampling, duplicate samples of eight mussels (each) were collected. For baseline data 10 replicates were used. The mussels were cleaned, measured, shucked and homogenized. An aliquot of the homogenized sample was weighed (wet weight) and lyophilized using a Virtis Model 10-010 freeze drier; the freeze dried tissue was weighed again and designated as the dry weight. The wet-dry weight ratio was calculated by dividing the wet weight by the freeze dried tissue weight.

Trace Metal Analysis. Aliquots of freeze dried samples (0.8g), placed in an acid cleaned glass volumetric flask, were digested in 5 ml Ultrax concentrated nitric acid for six hours at $50^{o}C$ and then diluted to a final volume of 50 ml with deionized glass distilled water. The digested and diluted sample was filtered through a clean and pre-rinsed Millipore glass fiber filter to remove particulate materials which tend to block the aspirator during analysis.

Copper and zinc were analyzed by the conventional flame atomic absorption spectrophotometry using an Instrumentation Laboratory Model 151 Atomic Absorption Spectrophotometer. Cadmium and nickel were analyzed by the graphite furnace flameless atomic absorption spectrophotometry (Perkin Elmer Model 5000 AA and HGA 500 graphite furnace). Mercury was determined using a cold vapor flameless atomic absorption spectrophotometer (Coleman MAS-50) after reduction of oxidized mercury to Hg^{o} with stannous chloride. Results were corrected for reagent blanks and calibrated by comparison with standard solutions of metal salts in 10% vol/vol nitric acid in DIDW. For quality control of the analytical results, similarly prepared samples of a standardized reference material (NBS #1577 Bovine Liver or NBS #1566 Oyster Tissues) were analyzed for Cd, Cu, Ni and Zn and compared with certified values.

Throughout the analysis of trace metals, meticulous care was exercised to minimize contamination, particularly in cleaning laboratory glassware and plastic ware. All glassware was washed with Acationox, rinsed with tap water, followed by three rinses with glass distilled water; these were soaked overnight in 50% nitric acid made up in glass distilled water, rinsed three times with deionized distilled water and once with 2% Ultrax nitric acid in deionized distilled water, followed by three rinses with deionized water. Plastic ware was immersed in acetone and rinsed three times with tap water to remove plasticizers and other soluble residues from the manufacturing process (Gill, 1980). After this pretreatment, the plastic ware was cleaned following the procedure outlined for the glassware.

Statistical Analysis of the Data. In the interpretation of the results, it is desirable to separate, though often with difficulty, the effects of normal physiological activities from those which are truly ascribable to perturbations resulting from anthropogenic activities on the uptake of trace metals in mussels. Implicit also in the field experiment is the fact that the data set is correlational and causation cannot be assumed; it is unlike laboratory experimentation where independent variables can be carefully controlled and altered one at a time,and the responses of the organism to them (e.g., uptake of metals) accurately measured.

Two issues are addressed in the analysis of mussel trace metals data. We first identify factors: wet/dry ratio, length of mussels, dredge volume, river runoff and temperature, which singularly or in combinations could be correlated to metal concentrations in mussels collected from four sites. Secondly, we determine whether metal concentrations in mussels differed among the four stations: D1, D2, D3 and ND.

The independent variables investigated in this study can be categorized in two groups, intrinsic and extrinsic factors. Intrinsic variables are associated with the physiological condition and growth of the mussels which are expressed as the wet/dry ratio of tissues and shell length, while extrinsic factors are variables in the environment, e.g., dredge volume, river runoff, water temperature that could influence metal concentrations in mussels. Since the purpose of this study is to determine whether extrinsic factors, especially disposal of spoils, are related to metal concentrations in mussels, it is extremely important that variance in metal concentration due to intrinsic factors be accounted for or removed. This is achieved by "weighting" metal concentration against the intrinsic factors. The method of weighting is explained in Fig. 4. One may view this manipulation as a form of data transformation when mussel metal concentrations are weighted by the intrinsic factors using the following equation:

$$y \text{ weighted} = (\bar{y}-\hat{y}_i) + y_i,$$

where y = dependent variable, $\bar{y}$ = mean of y, $\hat{y}$ = predicated y from the linear regression equation: $\hat{y} = a + bx$, y_i = the i'th value of y, a = y intercept, and b = slope of the regression line. The results of this weighting method are characterized by the fact that the manipulation renders (1) no change in $\bar{y}$ and in any x_i, (2) b = o in a regression of y weighted and x and (3) a reduction in the variance of y weighted as compared with that of y. This procedure was applied to all data sets. Aside from using correlation statistics to determine whether intrinsic and extrinsic variables were correlated, stepwise multiple regression analyses were performed on the weighted data to determine how much of the remaining variance can be explained by extrinsic variables which are entered into the regression model based on their significant partial correlation coefficients.

To determine whether metal concentrations in the mussels differed among sites, Friedman's randomized blocks test was used. If the test revealed a significant difference ($P<\cdot 10$), multiple comparison tests were performed to discern which sites contributed to the observed difference.

Results and Discussions

Relationship between Metal Concentrations and Intrinsic Factors

It is reported that trace metal concentrations in shellfish are influenced by their reproductive state and the size of the organisms (Boyden, 1974, 1977; Wilt, 1974; Romeril, 1974; Behrens and Duedall, 1981). The annual cycle of wet/dry weight ratios,which represent the building up and depletion of glycogen in the mussel tissue, is shown in Fig. 5 for the mussels maintained at the four monitoring stations. The low ratios occur during March, April, May and June representing prespawning conditions, while the subsequent rising ratios denote the onset of spawning and depletion of tissue reserves. The condition of the mussels deteriorates progressively during winter. In early spring, the mussels begin to replenish their depleted reserves, which is probably associated with heightened feeding activities during the seasonal diatom bloom.

On visual inspection of the results of Cd, Cu, Hg, Ni and Zn shown in Figs. 6, 7, 8, 9 and 10, trace metal concentrations appear to exhibit seasonality with peaks generally in December to February. It is logical to question whether the observed variations in W/D ratios, length of shell and metal concentrations are related. To this end correlation coefficients of the intrinsic variables with the five metals at four stations were determined (Table 1). Wet/dry weight ratios consistently showed positive correlation with tissue metal concentrations. Shell length was always positively correlated with metals at North Dumpling but negatively at D1; the correlation coefficients were either positive or negative at D2 and D3. Boyden (1974) reported an inverse relationship between the concentration of Cd and shell length in mussels. In this study, we found that this relationship is by no means consistent; this, however, could be the result of using mussels of a limited size range (4-7.5 cm) in the present study, which constrained the size-dependent variability in metal concentration. In general, wet/dry weight ratio was a better predictor than shell length for trace metal concentration.

Relationship between Metal Concentrations and Extrinsic Factors

Partial correlation analysis (Draper and Smith, 1966) was used to determine which extrinsic variables: dredge volume, river runoff and temperature (Fig. 11) were significantly correlated with trace metal concentrations, after weighting by intrinsic variables, as if each extrinsic variable was the only one considered. Three dummy extrinsic variables were created to seek factors which might affect trace metal concentrations in mussels. The variables "Spring" and "Fall" correspond to seasonal phytoplankton blooms during February, March and April as well as August and September respectively. The "Year" variable can be related to annual variations in runoff or dredge volume. It is important to examine all potentially significant extrinsic variables as will be seen in the following section. When stepwise multiple regression models are applied to account variance by the best combinations of extrinsic variables, one extrinsic variable can deny entry of another and alter the variance contributed by other extrinsic variables. This could conceal both of the extrinsic variables that were highly correlated with metal concentrations.

Tables 2 and 3 summarize the results of partial correlation analyses. Significant partial correlation coefficients ($P<0.05$) of trace metal concentrations with river runoff (R), temperature (T) and dredge volume (D) were detected six times, four times and one time respectively (Table 2). Runoff showed significant partial correlations with Cu, Ni and Zn at stations D1 and D2, but never with Cd and Hg. In the six significant correlations between runoff and metal concentrations, we found only one case in which runoff was inversely related to Zn at D2 (Table 4).

Temperature showed a significant partial correlation with the concentration of Cd and Cu in mussels at all four stations (Table 4). At D2, Cd was positively correlated with temperature, while at D1, D3 and ND Cu and Cd were inversely correlated with temperature (Table 4).

Only in one instance did we find a significant partial correlation between dredge volume (D) and Cd at D1; the dredge volume was not significantly correlated with any other trace metal or site (Tables 3 and 4).

A significant inverse relationship between years (Y) and the concentration of Hg and Cd was noted at D3 and ND, which suggests a decline in the concentration of these metals over the study period. However, the results may be affected by the small sample size available for the analyses at both sites. Spring (S) was significantly correlated with Hg and Cu at ND, suggesting that the concentration of these metals may be associated with an increased feeding of the mussels when the annual spring phytoplankton bloom occurs during February, March and April.

Mercury was the only metal which was not significantly correlated with the three principal extrinsic factors at any site.

The results reveal complex correlations existed between extrinsic factors and mussel trace metal concentrations. Interpretation of some of the negative partial correlations is difficult without further refinement and expansion of extrinsic factors. However, it is sufficient to say that the temporal variation of trace metal concentrations observed in mussels was most frequently correlated with runoff but not with dredge volume. This finding is probably not surprising due to the close proximity of the disposal site to the river which perhaps has a more pervasive year-round influence on the site than the episodic dredging and disposal activities.

Variance in Trace Metal Concentration Associated with Groups of Variables (Stepwise Multiple Regression Analysis).

Having established that the intrinsic and extrinsic factors are important determinants of trace metal concentrations observed in mussels, the next logical question we ask is: to what extent do these variables contribute to the variance of metal concentrations at each station? Thus, stepwise multiple regression analyses were performed on the weighted metal concentrations in mussels, intrinsic and extrinsic variables. As a rule the intrinsic variables, wet/dry weight ratio and shell length are always entered in the model first to weight the metal concentrations and extrinsic variables. The order by which intrinsic factors enter the models reflects the degree of correlations exhibited by the variables. To wit, for Ni wet/dry weight ratios with greater correlation coefficients than that of the shell length measurements, enter the model first at D1, D3 and ND (Table 1). Similar considerations, i.e., the magnitude of the partial correlation coefficients, govern the order of extrinsic variables entering into the regression models. For example, for Cu at ND runoff has the highest partial correlation after wet/dry ratio and shell length, hence enters the model. This procedure is used to determine the "best" regression model that could explain most of the variance in the trace metal concentration at each site. Furthermore, within each model the R^2 can be used to assess which variable contributes the most variance in the metal concentration.

The results are presented in Table 4. Thirty percent of the "best" models consists of only two intrinsic variables, the minimum possible number, whereas the largest model Cu at D3, has seven variables and accounts for 97% of the variance in the metal concentration. This observation strongly suggests that, in most cases (70%) tissue trace metal concentrations are affected simultaneously by a number of intrinsic and extrinsic factors. Dredge volume entered the Cd, Cu and Zn models at some sites but not the Hg or Ni model. For some metals (Zn, Cu, Cd) dredge volume entered models at D1, D2 and D3, but not at ND. This may suggest that there is a gradient effect of dredge spoil disposal along the stations, since ND is farthest from the dumpsite. Also at D1, dredge volume was the first and the only extrinsic variable in the Cd model, and entered after runoff in the Zn model; it explains 31% (R^2) and 7.8% of the variance in the concentration of Cd and Zn respectively. Furthermore, dredge volume has a positive partial correlation with Cd and Zn at D1. All these observations seem to support the gradient effect of dredge volume mentioned above.

Among the extrinsic variables, runoff enters 35% of the models. It is frequently the first extrinsic variable to enter the models, which is consistent with the result obtained by the partial correlation analysis reported in the previous section. It is conceivable that runoff or suspended materials carried with it could have increased the availability of trace metals in the environment, and in turn affected Cd, Cu, Ni and Zn concentrations in the mussels. Runoff was never entered into the Hg model at any site.

The entrance of temperature as an extrinsic factor into the models, in all likelihood, is based on the fact that it is the major driving force of a number of physical and biological processes which in turn can influence metal concentrations in the mussels. It enters in 20% (four of the twenty)of the models. It is the first extrinsic variable to enter the Cd model at D2, Cu model at D3, Cu model at ND and the last to enter Ni model at D3.

"Months" and "Years" entered in five of the 20 models, four cases at D3 and one case at ND. "Years" was the first extrinsic variable to enter the models at ND for Cd and at D3 for Hg; the negative partial correlations between years and the metals at these sites indicate a decline of metal concentrations at these sites from 1977-1979.

Dummy variables (Spring, Fall, Amino) entered four models and were used to explore future areas of research. For example, Hg at ND shows a significant partial correlation with "Spring". Conversely, Cd at D3 is partially correlated with "Fall". Hence, the "Spring" and "Fall" extrinsic variables may be associated with the spring and fall phytoplankton blooms which in turn could influence the level of trace metals in the mussels through heightened feeding activities.

Table 5 summarizes the total percent variance in trace metal concentration accounted for by the intrinsic and extrinsic variables in the 20 stepwise multiple regression models. The percentage of variance explained ranges between 14-97% with median of 61.4%. It is noticed that in 30% of the cases (6 of the 20) variance in the metals is explained by intrinsic variables only; they are Hg at D1 and D2, Ni at D1 and ND, and Zn at D3 and ND.

In the main, the results of this section confirm the conclusions reached by the partial correlation analysis. Runoff enters the models more often (7 times) than the Dredge Volume (4 times) and Temperature (4 times) and is frequently the first extrinsic variable to appear in the model (Table 6).

Comparison of Metal Concentrations and Wet/Dry Weight Ratios among Stations

In seeking answers to the question whether trace metal concentrations and wet/dry weight ratios differ among stations, Friedman's randomized blocks test was applied to analyze the data set (Noether, 1976). The test uses ranked paired variables (metal concentrations, W/D ratios) within randomized blocks, i.e., the months, and compares the rank sums among sites. We found that three trace metals, Cu, Hg and Ni as well as the wet/dry weight ratios were significantly different among sites ($P = .10$) (Table 7). The next stage of the analysis was to determine which rank sums are different among sites at $P \leq .1$; a multiple comparison procedure outlined in Noether (1976) is followed. The results are presented in Table 8. There was no significant difference in the concentrations of Cu for stations ND, D3 and D2, nor any among D3, D1 and D2; a significant

? Qualify

difference was, however, noted between stations ND and D2. Similar results were found in Hg concentrations and wet/dry weight ratio. No significant difference was detected in the concentrations of Ni and Zn among stations. However, there was a trend indicating that ND had lower concentrations of Cu, Hg, Ni and Zn, and lower wet/dry weight ratios than other sites. It is tempting to speculate that the lower metal concentrations in mussels at ND are due to the remoteness of this station from the disposal site. The significantly better condition of the mussels as shown by the lower W/D ratio at ND appears to support this interpretation. Furthermore, dredge volume which entered in other stations was never entered into the multiple regression models at ND. However, such an interpretation may be weakened due to the fact that the mussels at ND were collected from the natural substrate, unlike those at other sites, which were held in mesh bags.

Conclusions

1. The variance of metal concentrations attributable to the intrinsic variable, Wet/Dry weight ratio, ranged from 4 to 81% with a medial of 43%.

2. Partial correlation analyses revealed that <u>runoff</u> was the most often correlated with trace metal concentrations.

3. Stepwise multiple regression analyses also showed that <u>runoff</u> entered the models more frequently than any other extrinsic variables. Dredge volume entered four metal models which were associated with the stations located in or near the disposal site. Relatively speaking, dredge spoil disposal was probably not a major factor in the increased uptake of trace metals by the mussels at the New London dump site.

4. Temporal related variables, spring, fall, months and years entered seven of the models, signifying the existence of annual and seasonal variations in trace metals at ND and D3.

5. There were significant differences ($P \leq \cdot 1$) in wet/dry weight ratios and in Cu, Hg and Ni concentrations among sites. North Dumpling (ND), located farthest from the disposal site, appeared to have the lowest metal concentrations and most favorable wet/dry weight ratios than any other stations.

References

Arimoto, R. and S.Y. Feng, 1982. Changes in the levels of PCBs in Mytilus edulis associated with dredge spoil disposal. DAMOS Contribution No. 10. In: Waste in the Ocean, Vol. II: Dredged Material Disposal. D.R. Kester, I.W. Duedall, B.H. Ketchum, and P.K. Park, eds. John Wiley and Sons, Inc., New York, N.Y. (In press).

Behrens, W.J. and I.W. Duedall, 1981. Temporal variations of heavy metals in Mercenaria mercenaria. J. Cons. Int. Explor. Mer., 39, 219-222.

Boyden, C.R., 1974. Trace element content and body size in molluscs. Nature, 251, 311-314.

Boyden, C.R., 1977. Effect of size upon metal content of shellfish. J. Mar. Biol. Ass. U.K., 57, 675-714.

Bryan, G.W., 1971. The effects of heavy metals (other than mercury) on marine and estuarine organisms. Proc. Roy. Soc. Lond., 177, 389-410.

Calabrese, A., F.P. Thurberg and E. Gould, 1977. Effects of cadmium, mercury, and silver on marine animals. Mar. Fish Rev., 39, 5-11.

Draper, N.R. and H. Smith, 1966. Applied regression analysis. John Wiley and Sons, Inc., New York, N.Y.

Feng, S.Y. and G.M. Ruddy, 1975. Concentrations of zinc, copper, cadmium, manganese and mercury in oysters (Crassostrea virginica) along the Connecticut coast. The 3rd Internat'l. Ocean Develop. Conf., 4, 109-129. Aug. 4-8, 1975, Tokyo.

Fitzgerald, W.F., R.J. Szechtman and C.D. Hunt, 1974. Chapter II, A preliminary budget for Cu and Zn in Long Island Sound. pp 14-42. In: Investigations on concentrations, distributions and fates of heavy metal wastes in parts of Long Island Sound. Final Rpt. submitted to the Office of Sea Grant Programs, NOAA, Dept. of Commerce, October 1974. 161 pp.

Frazier, J.M., 1976. The dynamics of metals in the American oyster (Crassostrea virginica. II. Environmental effects. Chesapeake Sci., 17(3), 188-197.

Gill, G., 1980. Personal communication.

Goldberg, E.D., V.T. Bowen, J.W. Farrington, G. Harvey, J.H. Martin, P.L. Parker, R.W. Risebrough, W. Robertson, E. Schneider and E. Gamble, 1978. The mussel watch. Environmental Conservation 5(2), 101-125.

Greig, R.A. and J. Jones, 1976. Nondestructive neutron activation analysis of marine organisms collected from ocean dump sites of the middle eastern United States. Arch. Environm. Contam. Toxicol., 4, 420-434.

Greig, R.A., D.R. Wenzloff, A. Adams, B. Nelson and C. Shelpuk, 1977. Trace metals in organisms from ocean disposal sites of the middle eastern United States. Arch. Environm. Contam. Toxicol., 6, 395-409.

Leatherland, T.M. and J.D. Burton, 1974. The occurrence of some trace metals in coastal organisms with particular reference to the Solent region. J. Mar. Biol. Ass. U.K., 54, 457-468.

Nisbet, I.C.T. and A.F. Sarofin, 1972. Rates and routes of transport of PCBs in the environment. Environ. Health Perspect., 1, 21-38.

Noether, G.E., 1976. Introduction to statistics. Houghton, Mifflin Co., Boston, MA.

Phelps, D.K. and W.B. Galloway, 1980. A report on the coastal environmental assessment stations (CEAS) program. Rapp. P.-v. Réun. Cons. Int. Explor. Mer., 179, 76-81.

Pringle, B.H., D.E. Hissong, E.L. Katz and S.T. Mulawka, 1968. Trace metal accumulation by estuarine molluscs. Proc. Am. Soc. Civil. Eng., J. Sanitary Eng. Div., SA 3, 455-475.

Romeril, M.G., 1974. Trace metals in sediments and bivalve molluscs in Southampton water and the Solent. Rev. Intern. Oceanogr. Med., 33, 31-47.

Shuster, C.N. and B.H. Pringle, 1968. Effects of trace metals on estuarine molluscs. Proc. 1st Mid-Atlantic Industrial Waste Conf., Univ. Delaware, CE-5, 285-304.

Wilt, D.S. (Ed.), 1974. Eighth national shellfish sanitation workshop. U.S. Dept. HEW, PHS, Washington, D.C., 158 pp.

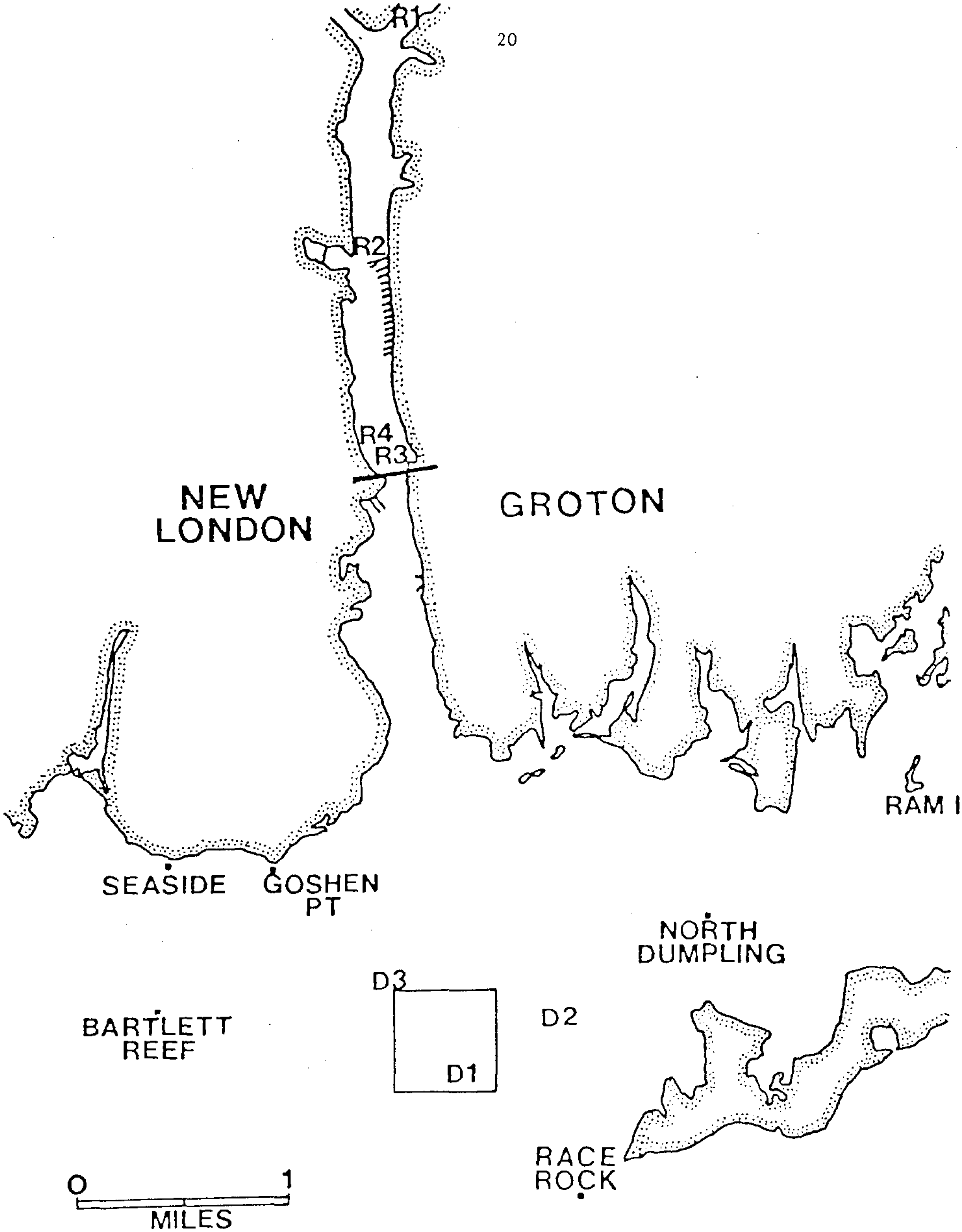


Figure 1. DAMOS New London disposal site and mussel monitoring stations: D1, D2, D3, and ND (North Dumpling).

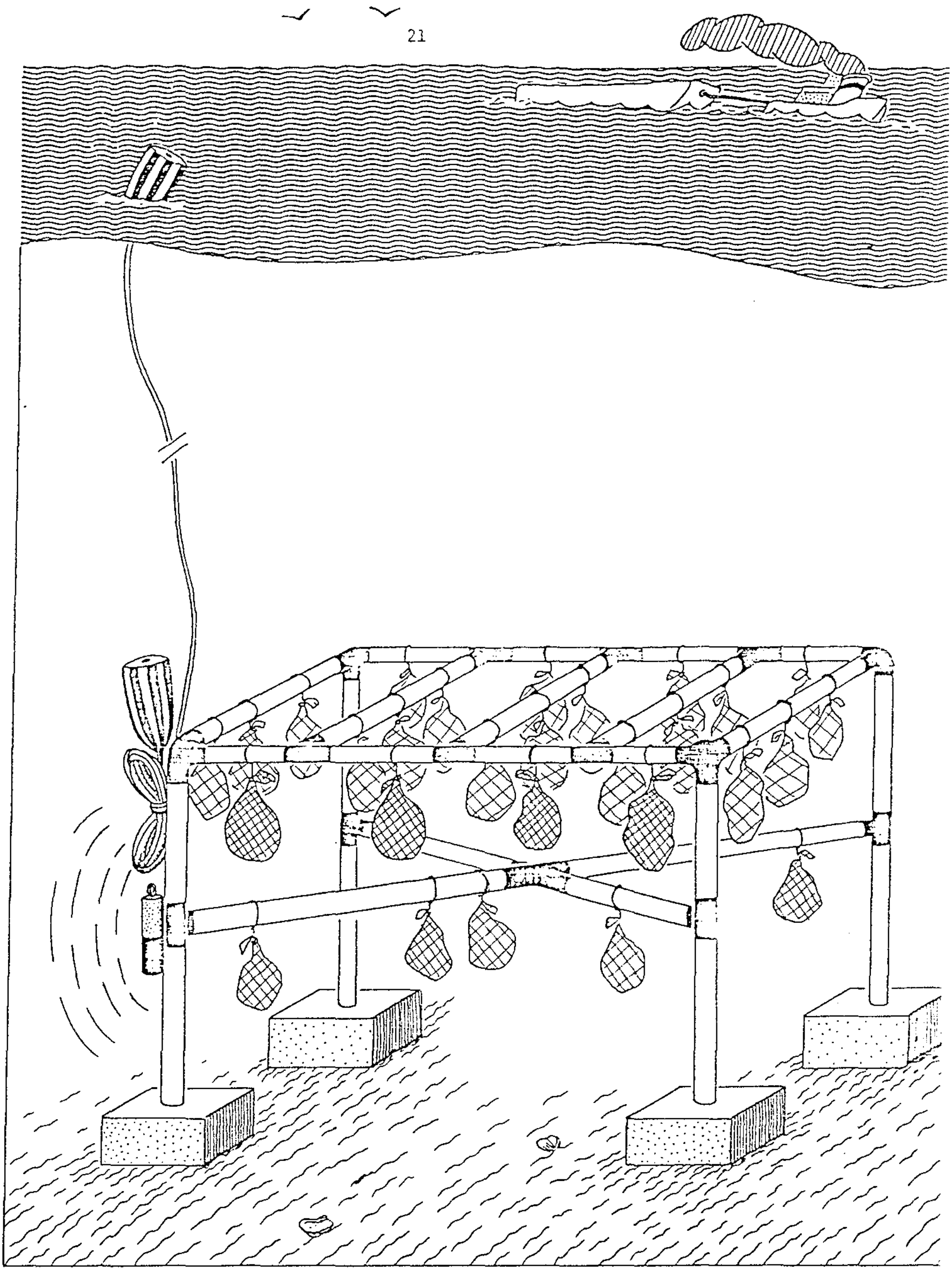
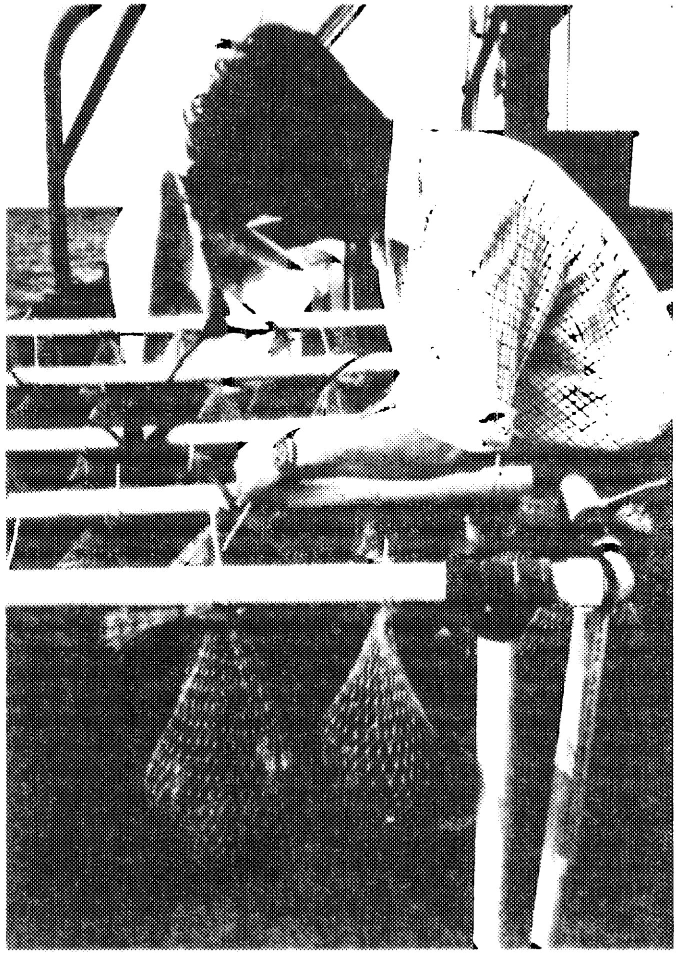
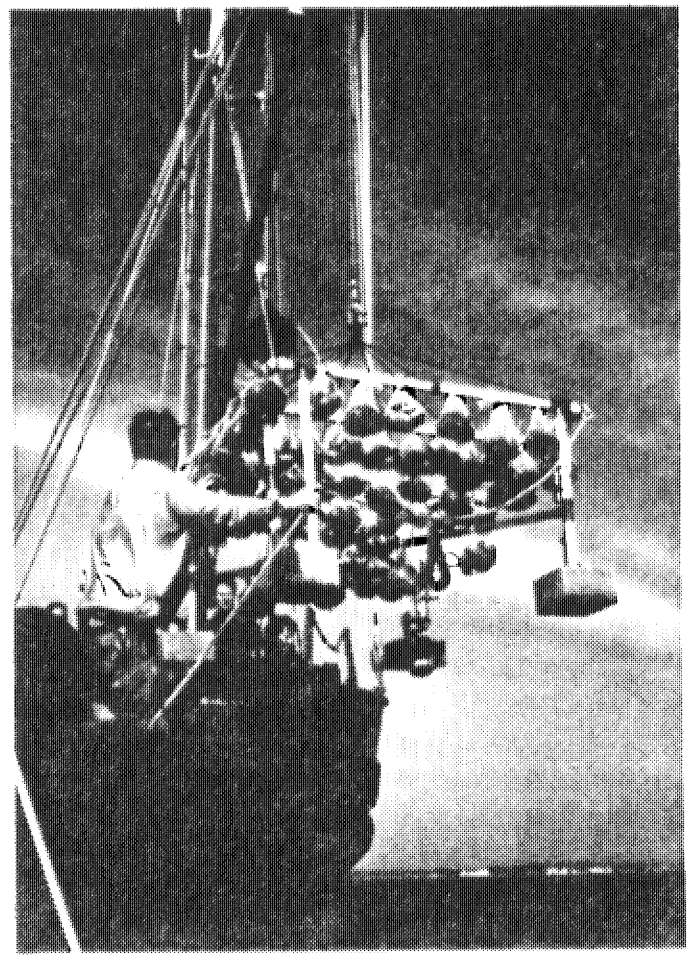


Figure 2. PVC platform used in maintaining shellfish at various monitoring stations.

Figure 3. Deployment of mussel monitoring platform in the field



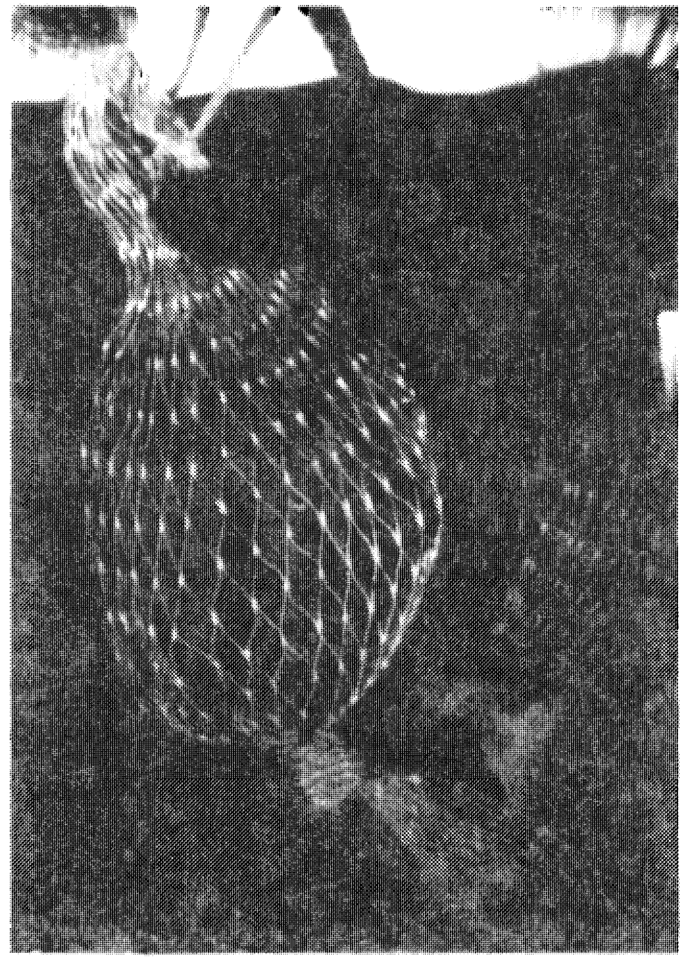
a. Attaching bags of shellfish to the the cross members of the platform with electric tie wraps.



b. A platform being lowered into the water.

Figure 3, Continued

c. Mussel platform resting on the seafloor showing the associated riggings.

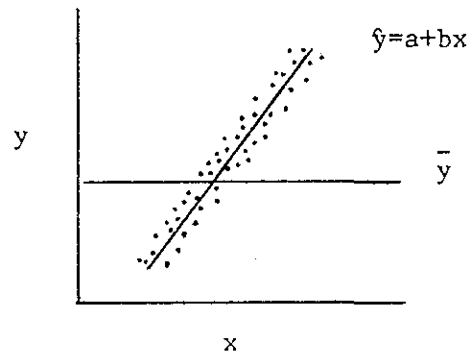


d. a close view of two bags of mussels suspended from a cross member of the platform.

Let:

x= independent variable
y= dependent variable
$\bar{y}$= mean of y
$\hat{y}$= predicted y from regression equation
y_i=the i'th value of y
a= the y-intercept
b= slope of the regression line

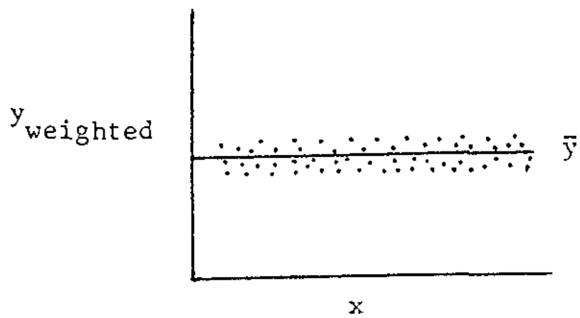
Hypothetical plot of x and y:



To weight y by x:

$$y_{weighted} = (\bar{y} - \hat{y}_i) + y_i$$

Plot of $y_{weighted}$ and x:



Weighting causes:

1) no change in $\bar{y}$
2) no change in any x_i
3) b=0 in a regression of $y_{weighted}$ and x
4) a reduction in the variance (i.e. variance of $y_{weighted}$ is less than that of y)

Figure 4. The method of weighting dependent variables (y_i) with intrinsic or independent variables (x_i).

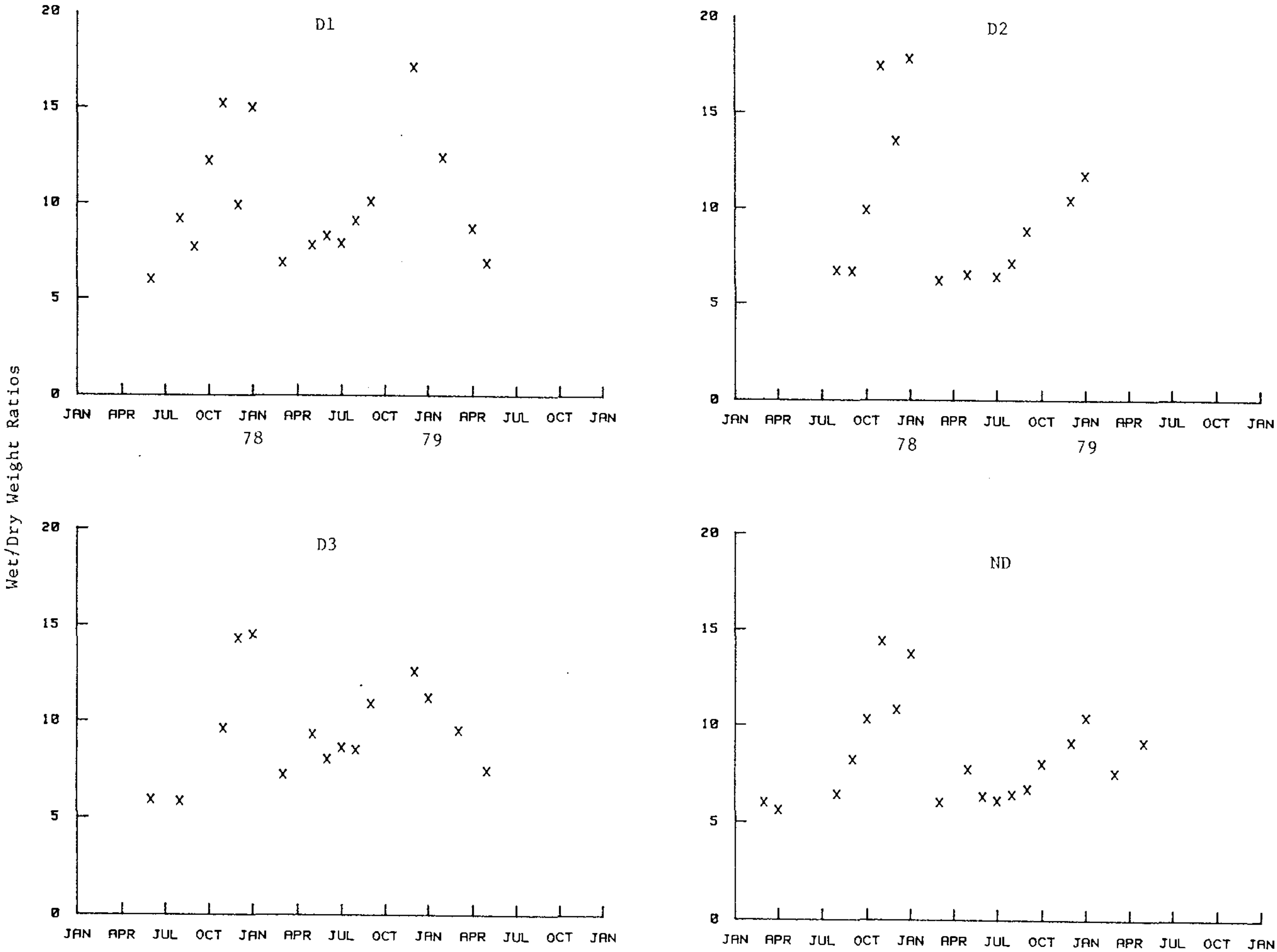


Figure 5. Temporal variations of mussel tissue wet/dry weight ratios at four monitoring stations.

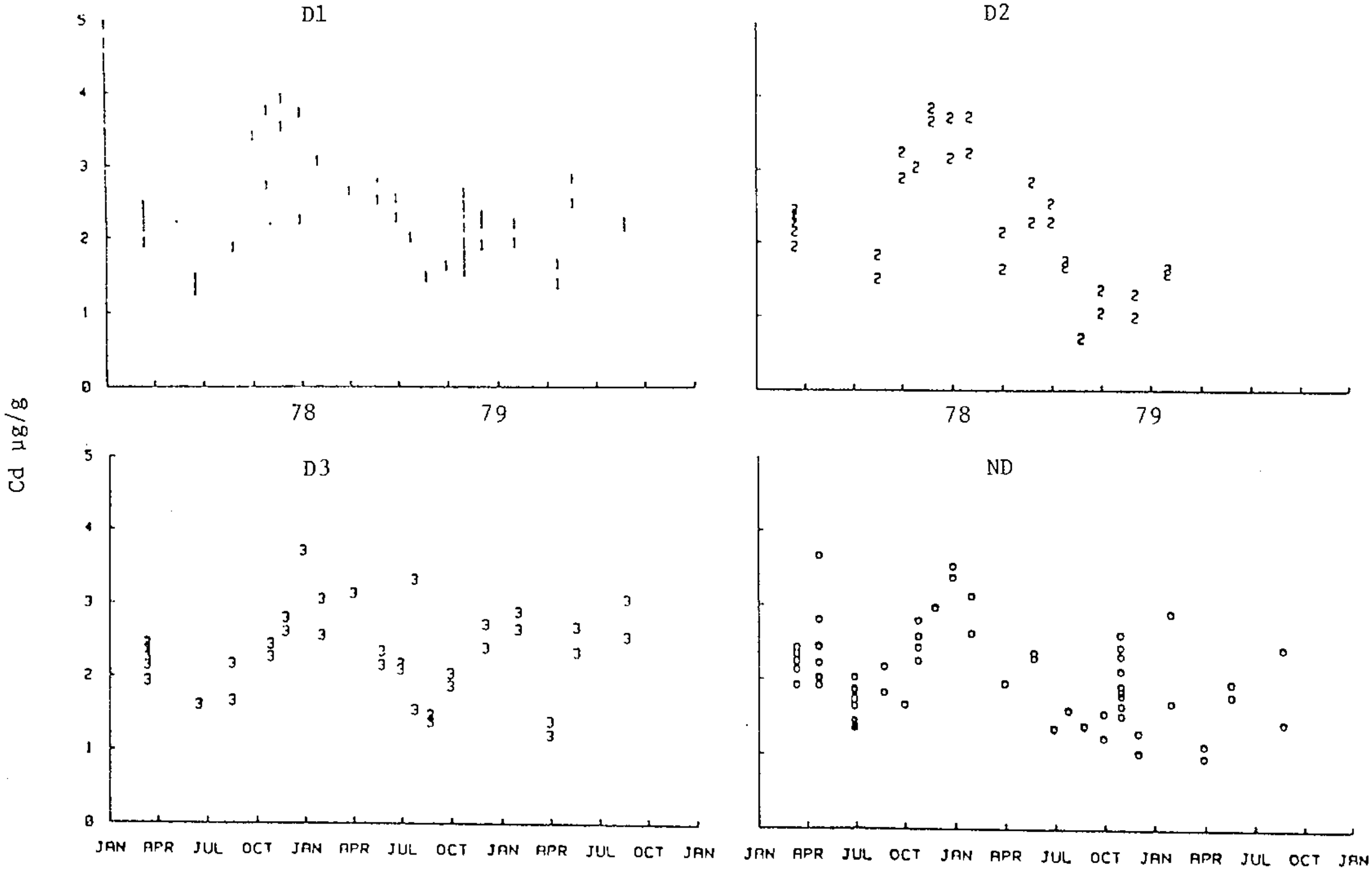


Figure 6. Temporal variations of cadmium concentration in _Mytilus edulis_ maintained at Stations D1, D2, D3, and ND.

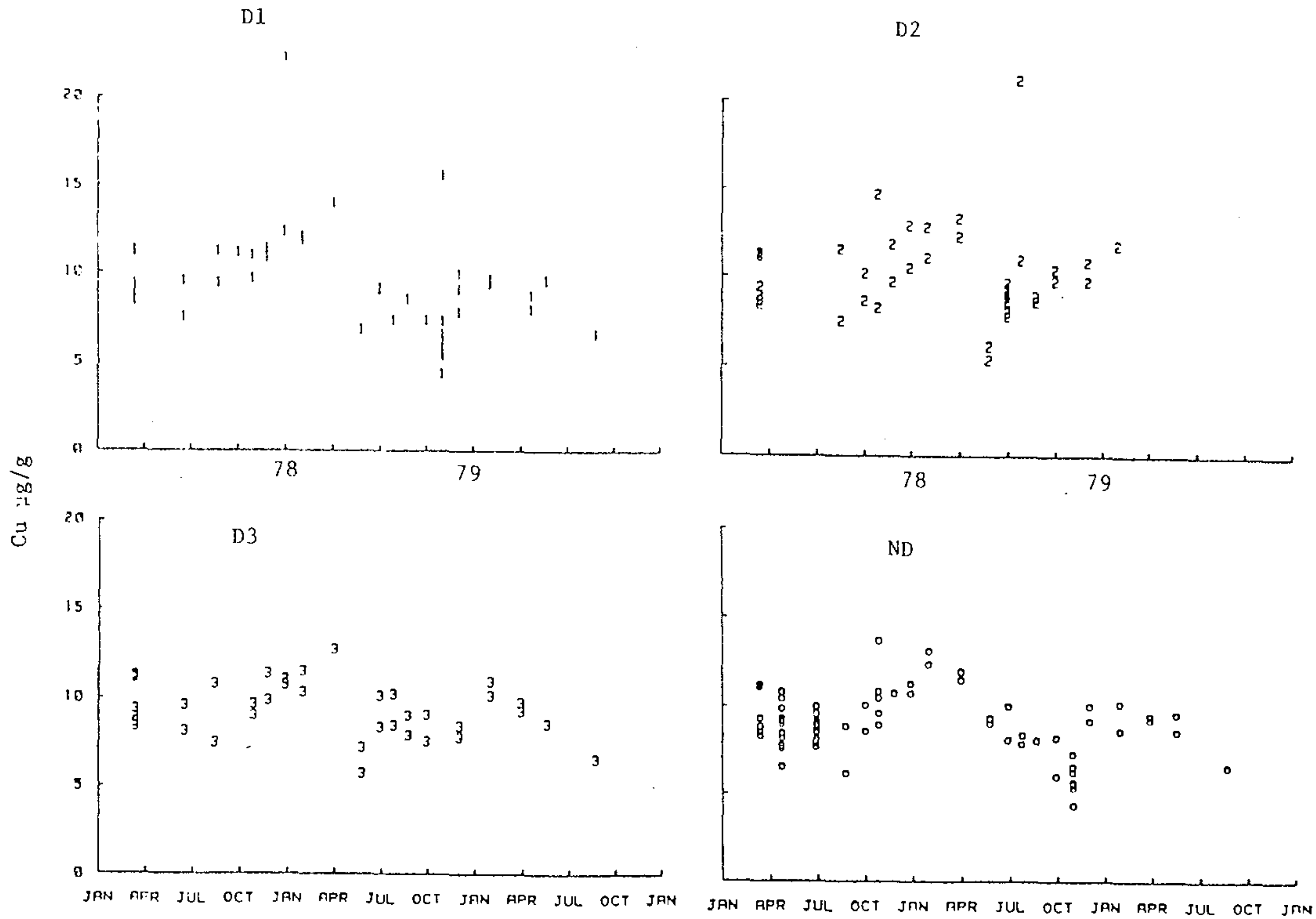


Figure 7. Temporal variation of copper concentration in *Mytilus edulis* maintained at Stations D1, D2, D3, and ND.

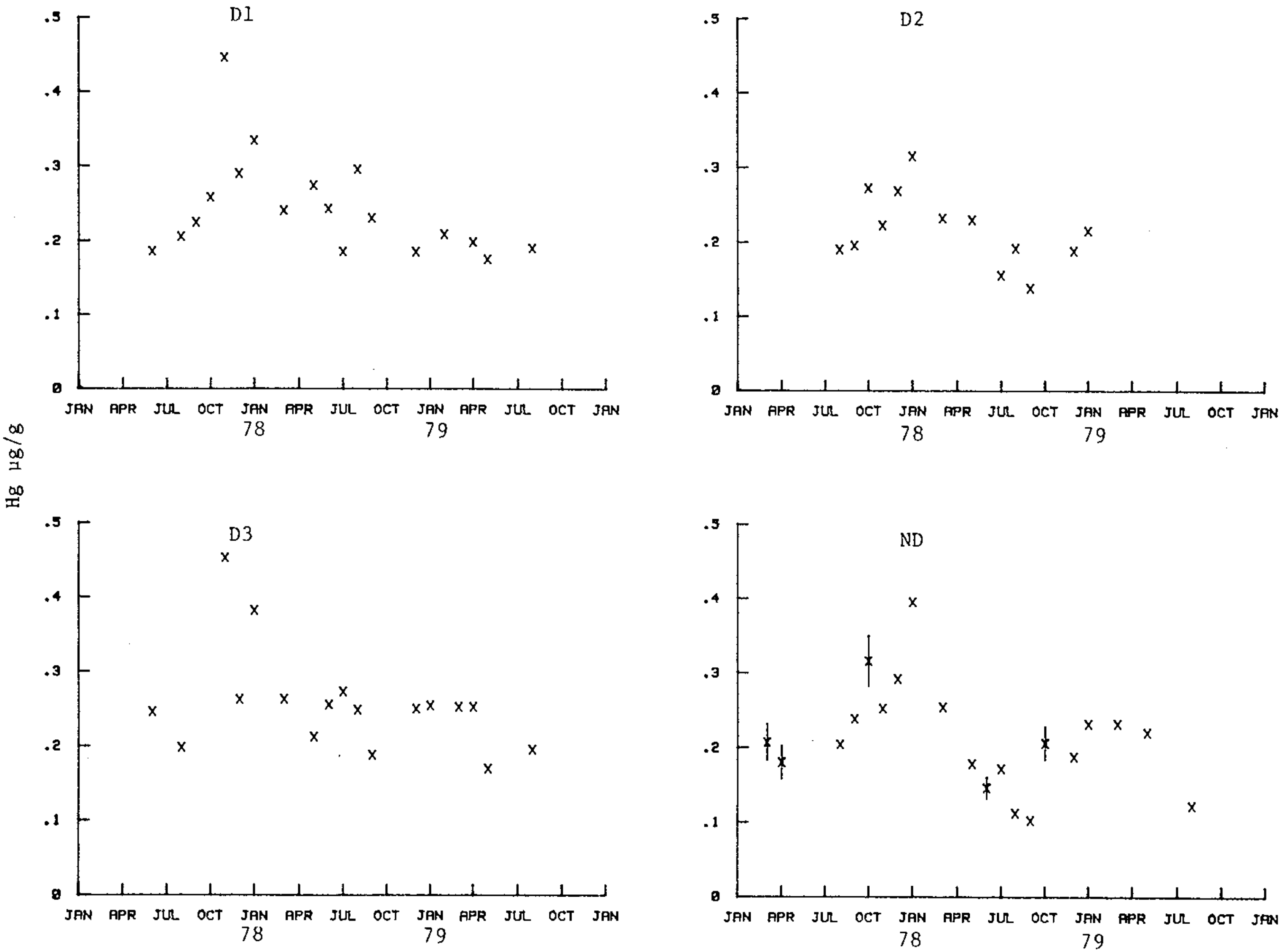


Figure 8. Temporal variations of mercury concentrations in *Mytilus edulis* maintained at Stations D1, D2, D3 and ND

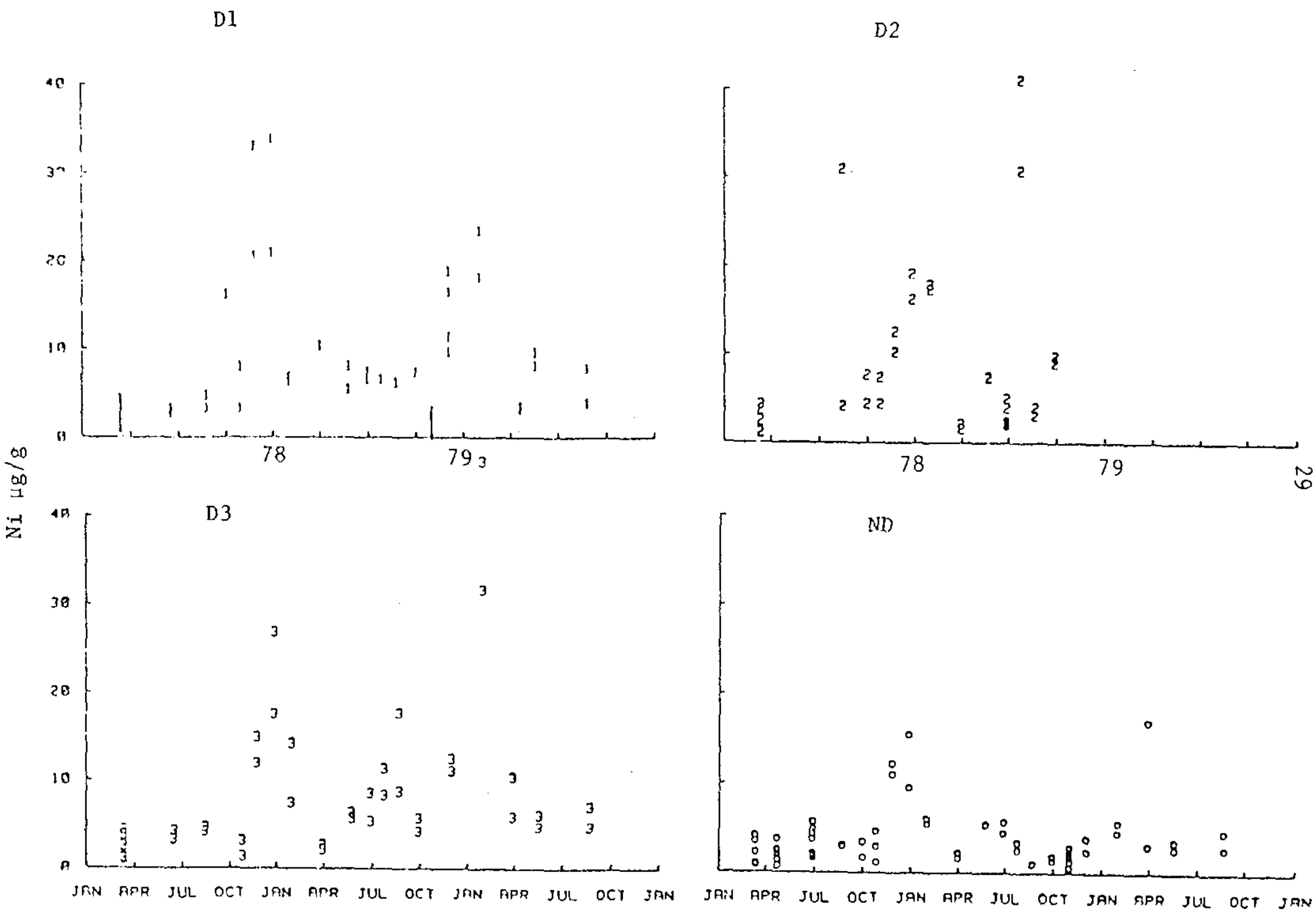


Figure 9. Temporal variations of nickel concentrations in Mytilus edulis maintained at Stations D1, D2, D3 and ND.

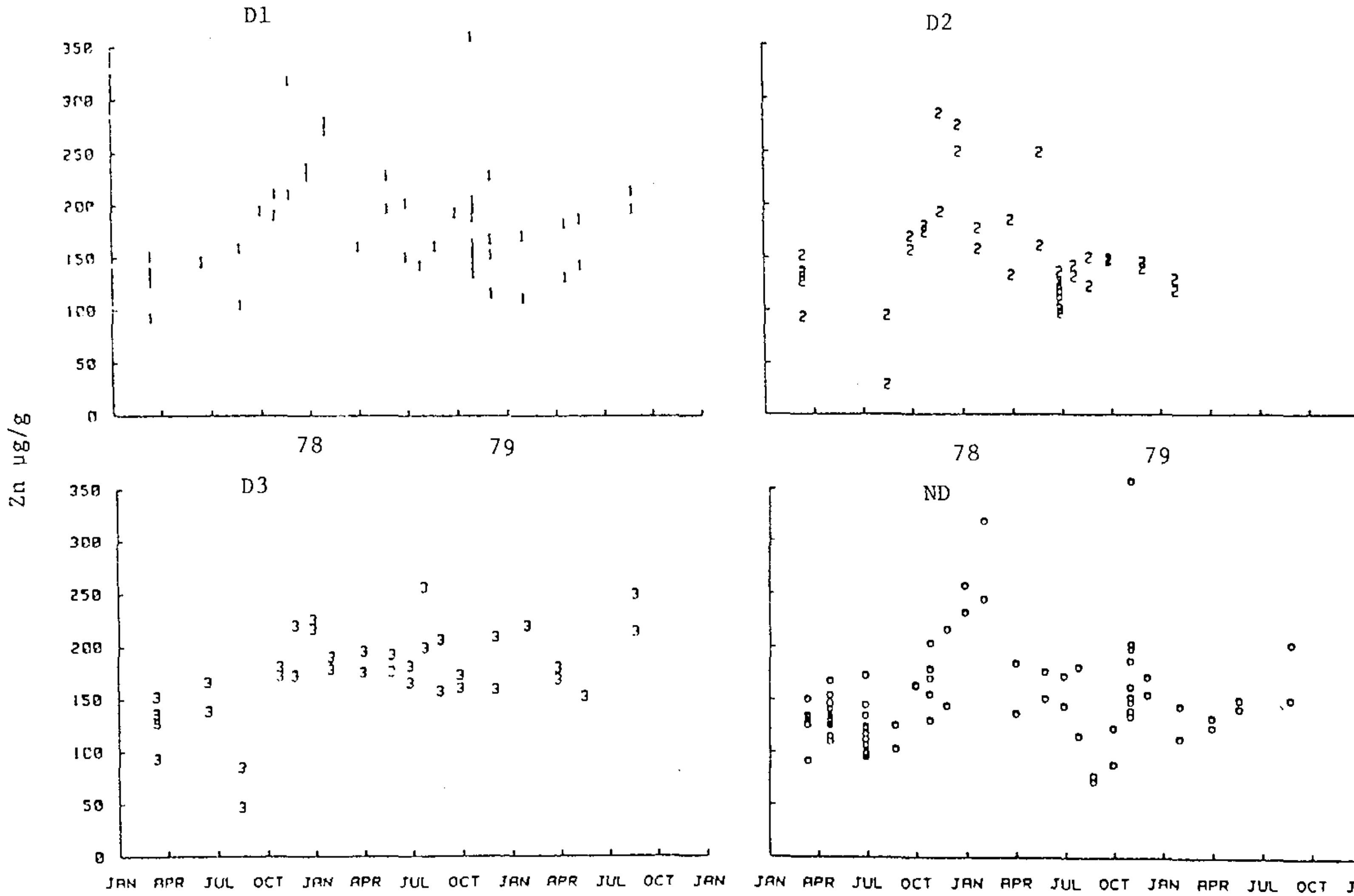


Figure 10. Temporal variations of nickel concentrations in Mytilus edulis maintained at Stations D1, D2, D3 and ND.

21

New London 77-79

Page 12: Good statement re: runoff vs dumping

It appears we may have settled the issue of impacts at New London. We should look at other sites more removed from riverine discharges.

pg 3, 3rd para - 2nd sentence is incomplete

pg's 8+9 Can't figure out their "weighting" formula. Can they further explain, in simple English? ~~Also, can't locate Friedman's randomized blocks test in any of our statistical books~~ - needs to be ~~explained so we can understand and use~~. OK, see Noether 1976 reference

pg 13 - What is R^2? Please define. Does it have something to do w/ runoff?

Table 4 - Appears that Station D4 should read "ND" (over)

pg 15, last para, ~~last~~ sentence – According to Table 8, there is no significant difference among sites ND, D3 and D1 for Cu. The first set of stations mentioned in this sentence should be changed accordingly.

p 16, 1st full sentence – This should qualify that there is a significant difference between sites D3 and D2, as well as D3 and ND.

pg16 – Why isn't there mention that Friedman's test shows no significant difference for Cd at any of the four sites tested?

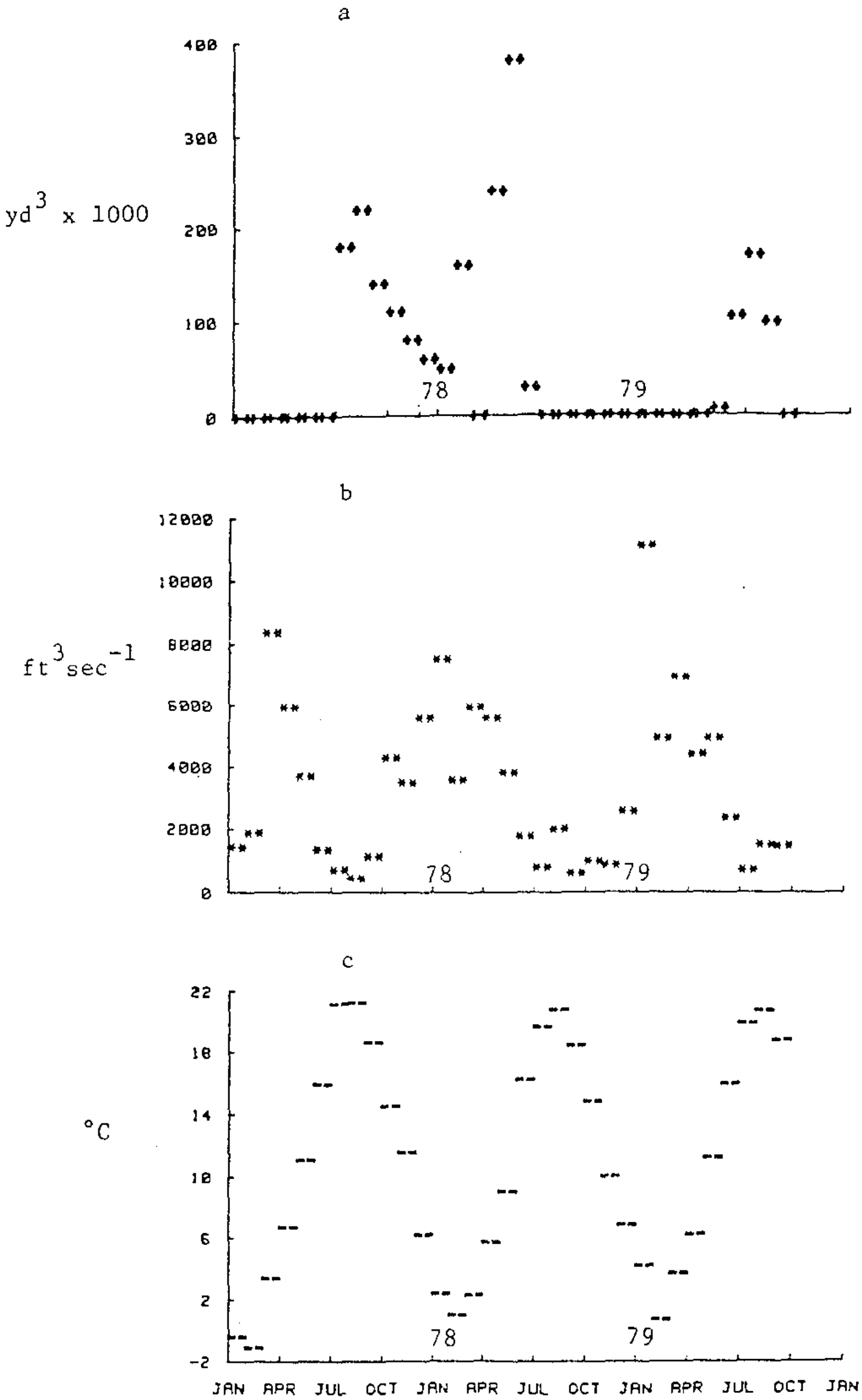


Figure 11. Temporal variations of three extrinsic variables: a. dredge spoil volume, b. runoff from Thames River and c. annual cycle of temperatures, °C.

METAL	INTRINSIC VARIABLE	STATIONS			
		D1	D2	D3	ND
Cd					
	W/D	+.328	+.618	+.484	+.604
	L	-.300	+.809	-.381	+.276
Cu					
	W/D	+.188	+.164	+.154	+.614
	L	-.295	-.315	-.141	+.050
Hg					
	W/D	+.476*	+.578	+.034	+.710
	L	-.378	+.718	+.373	+.199
Ni					
	W/D	+.438*	+.165	+.497*	+.640
	L	-.069	-.256	+.057	+.169
Zn	W/D	+.510	+.457	+.344	+.684
	L	-.441	+.727*	-.350	+.187

Table 1. Correlation coefficients of intrinsic variables, tissue wet/dry weight ratio and shell length with particular trace metals at the four stations. * denotes that the intrinsic variable was log(x) transformed to linearize its relationship with the trace metals.

METAL	EXTRINSIC VARIABLE							STATIONS			
	D	R	T	S	F	A	Y	D1	D2	D3	ND
Cd											
	X*							X			
			X						X		
							X				X
Cu											
		X						X			
		X									X
			X								X
				X							X
			X					X			
			X*							X	
Hg											
							X			X	
				X							X
Ni											
		X							X		
		X**								X	
Zn											
		X						X			
		X							X		

Table 2. A summary of all cases of significant extrinsic variables (partial correlation, $p<0.05$) for each trace metal at four stations. Read table by rows, e.g. dredge volume was significant to Cd concentration at D1. *=log(x), **=$(x)^2$. D = dredge volume, R = runoff, T = temperature of the water, S = spring, F = fall, Y = year, A = amino (dummy variable).

METAL	STATION	EXTRINSIC VARIABLE	n	P.C.C.	CRITICAL VALUE OF P.C.C.
Cd	D1	D	18	+.641	.497
	D2	T	13	+.710	.602
	ND	Y	19	-.661	.482
Cu	D1	R	18	+.642	.497
	ND	T	19	-.677	.482
	ND	S	19	+.629	.482
	ND	R	19	+.570	.482
	D1	T	18	-.559	.497
	D3	T	14	-.616	.576
Hg	ND	S	19	+.489	.482
	D3	Y	14	-.584	.576
Ni	D2	R	13	+.746	.602
	D3	R	14	+.693	.576
Zn	D1	R	17	+.567	.514
	D2	R	12	-.714	.632

Table 3. A list of the significant extrinsic variables, their partial correlation coefficients (P.C.C.) and the critical values for significance at the 0.05 level. n = sample size. The sign of the P.C.C. indicates the direction of the trend.

		STATIONS							
								ND	
METAL	VARIABLE	D1		D2		D3		D4	
			%		%		%		%
	1st	W/D	10.8	L	65.4	W/D	23.4	W/D	36.5
	2nd	L	24.3	W/D	81.5	L	31.6	L	37.5
Cd	3rd	D*	55.3	T	90.8	F	54.2	Y	64.9
	4th							R	75.6
	5th							A	81.2
			%		%		%		%
	1st	L	8.7	L	9.9	W/D	2.4	W/D	37.7
	2nd	W/D	14.9	W/D	17.0	L	3.6	L	39.6
	3rd	R	50.0	D	45.8	T*	40.2	T	67.3
Cu	4th	A	61.5			D	62.5		
	5th					Y	83.0		
	6th					R	92.7		
	7th					M	97.0		
			%		%		%		%
	1st	W/D*	22.7	L	51.5	W/D	13.9	W/D	50.4
Hg	2nd	L	46.3	W/D	66.5	L	15.4	L	50.5
	3rd					Y	44.2	S	62.3
			%		%		%		%
	1st	W/D	41.0	L	6.5	W/D*	24.7	W/D	19.2
	2nd	L	41.1	W/D	12.8	L	25.8	L	21.8
Ni	3rd			R	61.3	R**	61.5		
	4th					M	33.5		
	5th					T	93.4		
			%		%		%		%
	1st	W/D*	26.0	L**	52.8	L	12.3	W/D	46.7
Zn	2nd	L	57.0	W/D	61.3	W/D	14.4	L	46.8
	3rd	R	70.8	R	81.1				
	4th	D	78.6						

Table 4. Stepwise multiple regression models for Cd, Cu, Ni, Hg and Zn at four stations. % = the amount of variance in the trace metal explained by the model *after the variable on the same row has* entered the model. * = log(x), ** = $(x)^2$ transformation. Variable code: L = shell length, W/D = wet/dry tissue weight ratio, D = dredge volume, R = runoff, T = water temp., S = "spring," F = "fall," A = "Amino," Y = years, M = months.

METAL	STATIONS			
	D1	D2	D3	ND
Cd	55.3	90.8	54.2	81.2
Cu	61.5	45.8	97.0	67.2
Hg	46.3	66.5	44.2	62.3
Ni	41.1	61.3	93.4	21.8
Zn	78.6	81.1	14.4	46.8

Table 5. Total % variance in trace metal concentration accounted for by intrinsic and extrinsic variables and revealed by stepwise multiple regression models. One model was constructed for each trace metal at each station.

	EXTRINSIC VARIABLES							
METAL	D	R	T	S	F	A	Y	M
Cd	D1	ND	D2		D3	ND	ND	
Cu	D2,D3	D1,D3	D3,ND			D1	D3	D3
Hg				ND			D3	
Ni		D2,D3	D3					D3
Zn	D1	D1,D2						

Table 6. A summary of the extrinsic variables which entered the stepwise multiple regression models and were derived from Table 4. The table is designed to determine quickly which variables entered most often at particular stations.

VARIABLES	STATION	RANK SUM	FRIEDMAN'S STATISTIC	D.F.	SIGNIFICANCE
Cd	D1	27			
	D2	26			
	D3	28			
	ND	19			
			2.99	3	.39
Cu	D1	25			
	D2	33			
	D3	23			
	ND	19			
			6.24	3	.10
Hg	D1	26			
	D2	20			
	D3	34			
	ND	20			
			7.92	3	.05
Ni	D1	28			
	D2	29			
	D3	27			
	ND	16			
			6.60	3	.09
Zn	D1	26			
	D2	16			
	D3	28			
	ND	20			
			6.06	3	.11
Wet/Dry Ratio	D1	33			
	D2	31			
	D3	30			
	ND	16			
			9.87	3	.05

Table 7. Friedman's Test which compares the concentration of a trace metal and tissue W/D ratios among stations. Significance values close to or less than 0.10 suggest trace metal differences among station.

Cu

SITE	ND	D3	D1	D2
RANK SUM	19	23	25	33

CRITICAL
VALUE = 13.8

Ni

SITE	ND	D3	D1	D2
RANK SUM	16	27	28	29

CRITICAL
VALUE = 13.8

Hg

SITE	ND	D2	D1	D3
RANK SUM	20	20	26	34

CRITICAL
VALUE = 13.8

Zn

SITE	D2	ND	D1	D3
RANK SUM	16	20	26	28

CRITICAL
VALUE = 13.1

W/D Ratio

SITE	ND	D3	D2	D1
RANK SUM	16	30	31	33

CRITICAL
VALUE = 14.5

Table 8. Multiple comparisons tests to determine which of the sites differed significantly in the concentration of a single metal and wet/dry ratio. Sites connected by lines are not significantly different ($p \geq .10$). This test was only performed after the Friedman's test (Table 7) was significant, indicating that there was a difference among some sites. When the difference between any two rank sums is greater than the critical value then the rank sums are significantly different.