KELLY

MASTER OF SCIENCE THESIS

OF

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METAL CONCENTRATIONS OF SOME MACROBENTHOS OF NEW BEDFORD HARBOR AND AN EVALUATION OF THE USE OF THE SLIPPER LIMPET CREPIDULA FORNICATA LINNAEUS AS A MONITOR OF COASTAL METAL POLLUTION

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METAL CONCENTRATIONS OF SOME MACROBENTHOS OF NEW BEDFORD HARBOR AND AN EVALUATION OF THE USE OF THE SLIPPER LIMPET CREPIDULA FORNICATA LINNAEUS AS A MONITOR OF COASTAL METAL POLLUTION

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Metal pollution in coastal waters can have serious consequences to the marine flora and fauna and to man himself. The capability of a variety of marine organisms to concentrate trace metals from their environment has been demonstrated. Therefore, the use of certain species as monitors of metal contamination has been suggested.

New Bedford Harbor is a heavily polluted seaport. The copper, zinc and cadmium concentrations of the macrobenthos of the harbor area were analyzed by Atomic Absorption Spectrophotometry. Samples were collected from four stations with different sediment metal concentrations. Organisms were dredged from the harbor bottom with an epibenthic sled. Sampling emphasis was placed on the slipper limpet Crepidula fornicata in order to assess the potential of its use as a biological monitor of metal pollution.

There were no relationships between metal concentration and tissue weight in C. fornicata for copper, zinc or

cadmium. Crepidula fornicata exhibited significant positive correlations between tissue and sediment zinc and cadmium concentrations. Use of the slipper limpet to give [illegible] metal concentrations in the [illegible] no correlation between slipper limpet and sediment copper concentrations. Therefore, the role of C. fornicata as a monitor of copper pollution appears uncertain. Further study over a larger geographic area would help verify the monitoring status of C. fornicata.

The metal concentrations of two other molluscs and of four crab species were determined. There was some evidence of metal contamination in quahogs Mercenaria mercenaria from the study area.

BIOGRAPHICAL SKETCH

Brian Kelly was born on November 28, 1953, in New York City. He received his Bachelor of Science from Stonehill College, North Easton, Massachusetts in June, 1975. He is an active member of the Ecological Society of America, the American Society of Ichthyologists and Herpetologists, and the Forbush Bird Club of Worcester County.

In September, 1975, Mr. Kelly entered the Graduate School at Southeastern Massachusetts University, North Dartmouth, MA. He is currently a candidate for the Master of Science degree in Biology.

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INTRODUCTION

Metals in very low concentrations are part of the natural marine environment as the weathering products of rocks and are introduced to the sea via rivers or the atmosphere. Goldberg (1957) states that in open ocean waters, the natural levels of metals are 10.0 ug Zinc/liter (ug/liter=parts per billion (ppb)), 3.0 ppb Copper and 0.11 ppb Cadmium. Preston, Jefferies, Dutton, Harvey and Steele (1972) report 4.0-20.0 ppb Zn, 0.25-3.0 ppb Cu and 0.01-0.62 ppb Cd in British coastal waters. In estuarine areas, values as high as 199-320 ppb Zn, 16.5-43 ppb Cu and 1.8-7.5 ppb Cd in the Bristol Channel, England, are reported (Boyden and Romeril, 1974). Waters with concentrations of 17-525 ppb Zn, 1.9-107 ppb Cu and 0.08-6.0 ppb Cd are found in Spanish and Portugese estuaries (Stenner and Nickless, 1975).

Metals in the ocean may exist in particulate form, adsorbed onto a particle or colloid, or in solution as a hydrated ion, chelated with an organic compound, or complexed with an organic or inorganic ligand. The behaviour of the metal and its availability to the biota are greatly affected by its chemical state. In an experiment testing the accumulation of mercury from solution by the American oyster Crassostrea virginica, mercury from an organic compound, methyl mercury chloride, was assimilated faster than that from the inorganic salt mercuric chloride (Cunningham and Tripp, 1975a). The mussel Mytilus galloprovincialis accumulated tetravalent selenium (Se^{+4}) to a significantly greater degree than hexavalent selenium (Se^{+6})

from solution (Fowler and Benayoun, 1976a).

Bottom sediments behave as traps for metals, sequestering the metals from the overlying water or receiving them in the form of precipitates. Consequently, the sediments may become greatly enriched in metals, and may later release these pollutants to the water colum upon resuspension (Renfro, 1974; Valiela, Banus and Teal, 1974; Duinker, 1975). Conditions in the sediment determine the complex forms the metals take, and hence their availability to the biota or even to the overlying or interstitial waters. These sediment conditions include the pH, oxidation-reduction potential, amount of organic matter, nature and size distribution of particles, and the kinds of ligands available (Phillips, 1977a).

Most of the unnatural input of trace metals to the sea is associated with rivers, as these are where many major industrial and municipal sewerage outfalls are located (Bryan, 1971). As the fresh water mixes with the salt water, metals are lost from solution by precipitation, or to phytoplankton by adsorption (Phillips, 1977a). Consequently, trace metal pollution is primarily an estuarine and coastal problem, though by no means confined to these areas. The magnitude of the problem is increased by the fact that estuaries and adjacent coastal waters are important nursery grounds for a major portion of the commercially important finfishes and their food sources and are also the main sites of extensive shellfish beds.

Certain trace metals are known to be essential metabolites

of life systems, particularly in regard to the proper functioning of many enzymes (Williams, 1953; Brooks and Rumsby, 1965; Lehninger, 1970). Zinc is essential for the activity of carbonic anhydrase and alkaline phosphatase, both important enzymes in molluscan shell formation (Coombs, 1972, 1974; Ireland and Wootton, 1977). Iron is associated with the enzymes catalase and peroxidase, and manganese is a required cofactor of arginase. Hemocyanins, the oxygen carrying pigments of most gastropods, all cephalopods and of malacostracan crustaceans, require the binding of copper atoms (Gardiner, 1972). Copper is also an important constituent of the enzymes tyrosinase and cytochrome oxidase, the latter compound being the terminal protein of the electron-transferring cytochrome chain, which can react with oxygen (Lehninger, 1970). Cadmium has no known biological role (Pringle, Hissong, Katz and Mulawka, 1968). It may act as an enzyme inhibitor, however, and is toxic and cumulative in marine organisms.

The concentrations of the essential metals in the tissues must be very delicately balanced, as concentrations above a certain threshold value result in sublethal and even lethal effects. Pringle et al. (1968) consider an element as being toxic if it impairs growth, reproduction or metabolism of an organism when supplied above a given level. Metal toxicity may result from the inhibition of certain enzymes by the overabundant element, by the precipitation of proteins in the tissues by the metals (Pringle et al., 1968; Bryan, 1971), through errors in protein synthesis caused by mispairing of

nucleotide bases, and by the depolymerization of biological macromolecules by the metals (Eichhorn, 1975). Toxic effects of metals have been noted in the decreased survival and growth rates of bivalve larvae reared in metal-enriched waters (Boyden, 1975; Calabrese, MacInnes, Nelson and Mills, 1977) and in static toxicity bioassays (Pringle et al., 1968; Eisler, 1971, 1977a, b; McLusky and Phillips, 1975; Jones, Neville and Radlett, 1976; Eisler and Hennekey, 1977).

The extraordinary ability of marine biota to concentrate trace metals from the environment is well documented (Brooks and Rumsby, 1965; Segar, Collins and Riley, 1971; Eisler, Zaroogian and Hennekey, 1972; Bryan, 1973; Graham, 1973; Boyden, 1974; Cunningham and Tripp, 1975a,b; Ireland and Wootton, 1977). Concentration factors, expressed as (ug metal/g organism)/(ug metal/g water), commonly range from $1X10^3$ to $1X10^6$ power for trace metals in most organisms.

Marine organisms can concentrate metals by three principal means, absorption of metals from food, absorption/adsorption of dissolved metals across body/plant surfaces, and ingestion of particulate material containing adsorbed metals (Bryan, 1971). The relative importance of each of these routes in metal assimilation of an organism is dependent on several factors, such as the metal, species, and physicochemical and environmental variables. Macroalgae have been shown to accumulate metals primarily from solution (Bryan and Hummerstone, 1973a, 1977; Haug, Melsom and Omang, 1974; Young, 1975; Foster, 1976). Bryan and Hummerstone (1971, 1973b) demonstrated

the uptake of copper, zinc and cadmium across the body surface to be important in the polychaete Nereis diversicolor, but the amount assimilated from food was not calculated.

Undoubtedly, all three routes contribute some metals to the majority of animals, especially in filter-feeding species such as bivalves (Moore, 1971). In the mussel Mytilus edulis uptake from food was believed to be the most important source of lead (Schulz-Blades, 1974) and other metals (Pentreath, 1973; Phillips, 1976a). In the shrimp Lysmata seticaudata and the mussel Mytilus galloprovincialis, selenium accumulation via the food chain was more important than that from solution (Fowler and Benayoun, 1976a,b), as was true for cadmium accumulation in the euphausiid Meganyctiphanes norvegica (Benayoun, Fowler and Oregioni, 1974). Albalones Haliotis rufescens had extremely high tissue lead concentrations after being fed lead treated brown algae (Stewart and Schulz-Blades, 1976). Metal uptake by ingestion of inorganic particles by bivalves is significant (Preston et al., 1972). In oysters Crassostrea gigas, Boyden and Romeril (1974) found the relative importance of metal uptake from solution and from inorganic particulate matter to depend on the metal concerned, while Ayling (1974) reported that the assimilation of metals with particulate matter was more important in the same species.

Marine organisms can remove accumulated metals either (1) across the body surfaces (2) into the gut and voided with the faeces, or (3) in the urine (Bryan, 1971). The degree and particular route of tissue metal regulation varies with the

metal and the species considered. Decapod crustaceans appear to be able to regulate their body zinc levels. Crabs Carcinus maenas regulated zinc mainly across body surfaces, especially the gills. In lobsters Homarus vulgaris, however, urine zinc loss took the place of loss across the gills and the hepatopancreas had an active role in removing and storing excess zinc from the blood (Bryan, 1967a, 1968). The hepatopancreas is also active in zinc regulation in the crayfish Austropotamobius pallipes, where excess zinc is removed with the faeces (Bryan, 1967b).

A general pattern emerges wherein the kidney, gills and digestive gland/hepatopancreas show the highest rates of uptake and hence contain the greatest metal concentrations, while the gonads, mantle and muscle tissue take up metals more slowly and have lower metal levels (Schulz-Blades, 1974). This has been recorded for Mytilus edulis (Pentreath, 1973; Phillips, 1976a), the scallops Pecten maximus and Chlamys operularis (Bryan, 1973), oysters Crassostrea virginica (Pringle et al., 1968; Cunningham and Tripp, 1975a) and in several New Zealand bivalves (Brooks and Rumsby, 1965).

Environmental variables such as salinity, water temperature, and season, and individual size (weight) have been shown to affect the metal concentrations of an organism. Substantial seasonal variations in total metal concentrations and tissue metal distribution were noted in mussels Mytilus edulis (Pentreath, 1973; Phillips, 1976a), oysters Crassostrea gigas (Pringle et al., 1968), scallops (Bryan, 1973), American

oysters C. virginica (Frazier, 1975, 1976) and for the whelk Busycon canaliculatum (Betzer and Pilson, 1974). Significant metal concentration differences between sexes have been noted only for whole mussels Choromytilus meridionalis (Watling and Watling, 1976a) and for the gonads of Mytilus californianus (Alexander and Young, 1976).

The relationship of tissue metal concentration with animal weight was investigated in several species. Romeril (1971) found general increases in the concentrations of zinc, copper and iron with increasing weight of quahogs Mercenaria mercenaria. In a subsequent study, however, he demonstrated copper and iron to be negatively correlated with quahog tissue weight while zinc concentrations were positively correlated (Romeril, 1974). Smaller shellfish showed a faster uptake of metals in the laboratory than larger conspecifics (Cunningham and Tripp, 1975b; Schulz-Blades, 1974). Boyden (1974) noted that highest metal levels were usually found in the smaller individuals of several molluscan species. Watling and Watling (1976a,b) observed smaller mussels and oysters to have greater metal concentrations than larger conspecifics from the same location. Copper, zinc and cadmium concentrations were also negatively correlated with weight in the Sydney rock oyster (McKay, Williams, Kacprzac, Kazacos, Collins and Auty, 1975). No relation was found between metal concentration and size with copper in the whelk Busycon canaliculatum (Betzer and Pilson, 1974) or with zinc in Crassostrea virginica (Huggett, Bender and Slone, 1973). Phillips (1976a)

found the total zinc, copper, cadmium and lead contents of M. edulis to remain fairly constant year round, with weight changes related to the reproductive cycle producing much of the observed seasonality of metal concentrations; minimal weight in winter and early spring was coincident with maximum metal concentrations.

Temperature and salinity have been found to have some effect on the uptake of metals. Increases in temperature and decreases in salinity both increased cadmium uptake in four bivalve species in the laboratory (Jackim, Morrison and Steele, 1977). Selenium accumulation by Mytilus galloprovincialis was dependent on water temperature (Fowler and Benayoun, 1976a). Temperature and salinity had varied effects on the uptake of metals by Mytilus edulis, depending on the metal (Phillips, 1976a, 1977b).

Thus, due to the varying effects of environmental variables and size on trace metal uptake, caution must be used when evaluating apparent differences in trace metal concentrations among conspecifics from different locations. Investigators utilizing monitoring organisms for evidence of metal pollution should therefore consider these effects.

The use of water analyses to monitor metal pollution is beset with difficulties. It is expensive and large volumes of water are needed to attain sufficient metal concentrations for analysis. Multiple sampling is necessary to eliminate variations in metal concentrations with time, season, fresh water runoff and tides (Phillips, 1976b). The use of sediments is

also subject to error, according to local variations in sedimentation rates of particulate metal, particle nature, form and size (Phillips, 1977a), and in the amounts of organic matter present, as metal concentrations generally increase with total carbon in the sediments (Halcrow, Mackay and Thornton, 1973). In addition, water and sediment metal analyses provide little data on the biologically available metal fraction (Phillips, 1977a).

The use of marine organisms for environmental monitoring would allow the quantification of metals available for accumulation. The monitor would be one used 'to quantify relative levels of pollution by measurement of toxicant concentrations in its tissues' (Phillips, 1977a). Monitoring organisms would also permit time-integrated estimates of available metal concentrations to be made. They have already concentrated the metals from the environment, thus simplifying analysis and increasing its accuracy. The utilization of biological monitors would aid in the protection of both the marine ecosystem and man. Monitoring organisms could be used to: (1) record any significant increase in the biologically available metals over time (2) aid in the discovery of major sites of pollution (3) analyze the accumulation of trace metals in marine food chains (4) indicate the relative amounts of metals available to man from marine finfish and shellfish.

Darracott and Watling (1975) have outlined six basic tenets for a monitoring organism. It should be: (1) capable of concentrating the pollutant without lethality to the levels

encountered or anticipated (2) sedentary so as to be representative of the region where collected (3) abundant (4) of reasonable size for tissue analysis (5) of sufficient lifespan to allow the sampling of more than one year-class (6) easily sampled and hardy enough to survive laboratory conditions.

Haug et al. (1974) added three requirements for monitoring organisms. These were: (1) toleration of brackish water (2) existence of a high concentration factor to allow direct analysis (3) presence of some correlation between the metal concentration of the organism and the average metal concentrations in its surroundings.

Several attempts have been made to identify possible monitoring organisms. The limpet Patella vulgata in Israel (Navrot, Amiel and Kronfeld, 1974), the digestive gland of Mytilus californianus in the Southern California Bight (Alexander and Young, 1976) and the mussel Mytilus edulis in Australia (Phillips, 1976b) have been used in coastal water monitoring studies, and each showed increased concentrations of trace metals by urban and local industrial outfalls. The highest metal concentrations were found in the seaweeds Fucus vesiculosus and Porphyra umbilicalis and the limpet Patella vulgata in the East Irish Sea, coincident with the highest sea water concentrations reported (Preston et al., 1972). Zinc concentrations in soft tissue of English coastal barnacles have been correlated with estimated levels of zinc in sea water at different locales (Walker, Rainbow, Foster and Crisp, 1975). Schulz-Blades (1974) suggested the use of Mytilus

edulis as an indicator of lead pollution after he showed a constant rate of lead uptake in the mussel dependent on the lead concentration of the medium. Changes in the metal concentrations of limpets Patella sp. and dog whelks Nucella lapillus were observed over time after transferance of specimens from an unpolluted to a polluted site in English coastal waters (Stenner and Nickless, 1974). Bryan and Hummerstone (1977) traced silver particulate contamination in the Looe Estuary in England via the tissue concentrations of the deposit-feeding bivalves Scrobicularia plana and Macoma balthica.

The relationship between sediment and tissue trace metal concentrations has also been investigated in marine pollution studies. Copper and silver concentrations in the polychaete Nereis diversicolor were roughly proportional to the total sediment concentrations of these metals (Bryan and Hummerstone, 1971, 1977; Bryan, 1974). However, there was no relation between sediment and tissue zinc concentrations, and Bryan and Hummerstone (1973b) suggested that zinc is regulated by the worm. Boyden (1975) demonstrated a gradual concomitant decrease in metal concentrations of both oysters and sediments with distance from a pollution source. Mercenaria mercenaria had metal concentrations somewhat correlated with sediment concentrations, but the correlation was strong for zinc (Romeril, 1974). Copper showed no increase in the clam despite a significant increase in sediment levels, which was attributed either to the biological availability of the sediment copper or to regulation by the clam.

Frazier (1976) found that the relative enhancement of tissue trace metals in polluted oysters C. virginica over control oysters reflected the general pattern of metal contamination in the sediments, but the relationship between sediment and tissue concentration was not linear in the oyster. Pitar morrhuana from Rhode Island Sound had greater metal concentrations near electroplating plant outfalls, but clams at all stations significantly reflected only the manganese and zinc levels of the sediment (Eisler, 1977b). Huggett et al. (1973) found copper, zinc and cadmium concentrations in the sediments not correlated with those in oysters Crassostrea virginica.

This study investigated the trace metal content of the macrobenthos of the New Bedford Harbor area. Emphasis was placed on the slipper limpet Crepidula fornicata, a filter feeder, to ascertain the feasibility of its use as a monitoring organism for copper, zinc and cadmium pollution.

Filter feeding molluscs are exposed to all three main routes of metal accumulation, namely, that from food, solution, and ingestion of inorganic particulate material (Phillips, 1977a). Previous studies (Preston et al., 1972; Boyden and Romeril, 1974) indicate that ingestion of inorganic particles may be the most significant route of metal uptake in filter feeding bivalves. The gastropod C. fornicata employs the same food capturing mechanism as these bivalve filter feeders (Orton, 1912; Gardiner, 1972).

Sediment metal concentrations of the very fine silt-clay fraction (hereafter referred to as the clay fraction) as

determined by Summerhayes, Ellis, Stoffers, Briggs and Fitzgerald (1977) are those used in this study. It is documented that this sediment fraction forms a very mobile carpet over the New Bedford Harbor and Buzzards Bay bottom, an unconsolidated layer which is readily resuspended by tidal currents (Rhoads and Young, 1970; Summerhayes et al., 1977). It is believed that the clay sediment metal concentrations at different locations reflect to a substantial degree the relative amounts of biologically available metals to which the filter feeding slipper limpets are exposed.

New Bedford is an industrialized urban seaport with a population of over 100,000. Together with adjacent Fairhaven (population 20,000), the city's wastes have a significant impact on the Acushnet River Estuary.

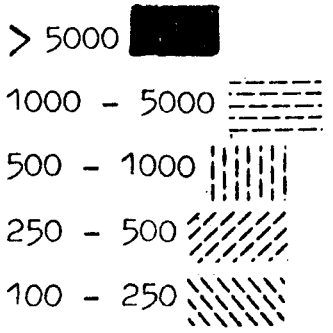
New Bedford Harbor is tidal and receives fresh water from the Acushnet River, the volume of this input being very small relative to the tidal volume of the harbor (Hoff, O'Brien and Cox, 1974). A hurricane barrier with a 150-foot wide/30-foot deep navigational opening, constructed by the Army Corps of Engineers in 1964-66, is located across the harbor entrance. Outside the barrier, the harbor is contiguous with Buzzards Bay.

Pollution of the Acushnet River Estuary is due to a combination of municipal and industrial wastes. Most sewage from New Bedford is processed through a primary treatment plant discharging effluent at Clark's Point outside the harbor at an average daily dry weather flow of approximately 114 million

liters (Summerhayes et al., 1977). Municipal and industrial wastes are also discharged at other areas into the inner harbor and Clark's Cove. Inner New Bedford Harbor waters have been classified as untreated sewage (Hoff, 1971). It is believed this hurricane barrier contributes to the problem by inhibiting tidal flushing and leads to a buildup of pollutants.

There is gross contamination of the Harbor with PCBs and trace metals (EPA, 1976; Army Corps of Engineers, unpbl. data). Summerhayes et al. (1977) found a definative gradient of metal concentrations in the very fine silt-clay fraction of New Bedford Harbor area sediments. This clay fraction was studied because, relative to the sand fraction, it was much enriched in trace metals. High concentrations of 5700-4000 parts per million dry weight (ppm) copper, 32-15 ppm cadmium and 1480-1100 ppm zinc were characteristic of the inner harbor sediments. Exponentially decreasing concentrations occurred in the sediments outside the harbor as distance from the hurricane barrier increased. Metal levels in these outer harbor sediments were 700-70 ppm copper, 8-1 ppm cadmium and 500-200 ppm zinc, from the barrier out into Buzzards Bay, respectively. Figure 1, showing the distribution of copper in this mobile clay fraction of harbor sediment, exemplifies this pattern. Figure 2 is a graphic summary of the sediment copper, zinc and cadmium concentrations, as determined by Summerhayes et al. (1977). Copper and zinc follow similar distribution gradients, while cadmium's dispersion is more complex.

Figure 1. Distribution of copper in the clay fraction of New Bedford Harbor sediment. All concentrations are in ppm dry weight. Redrawn from Summerhayes et al. (1977).



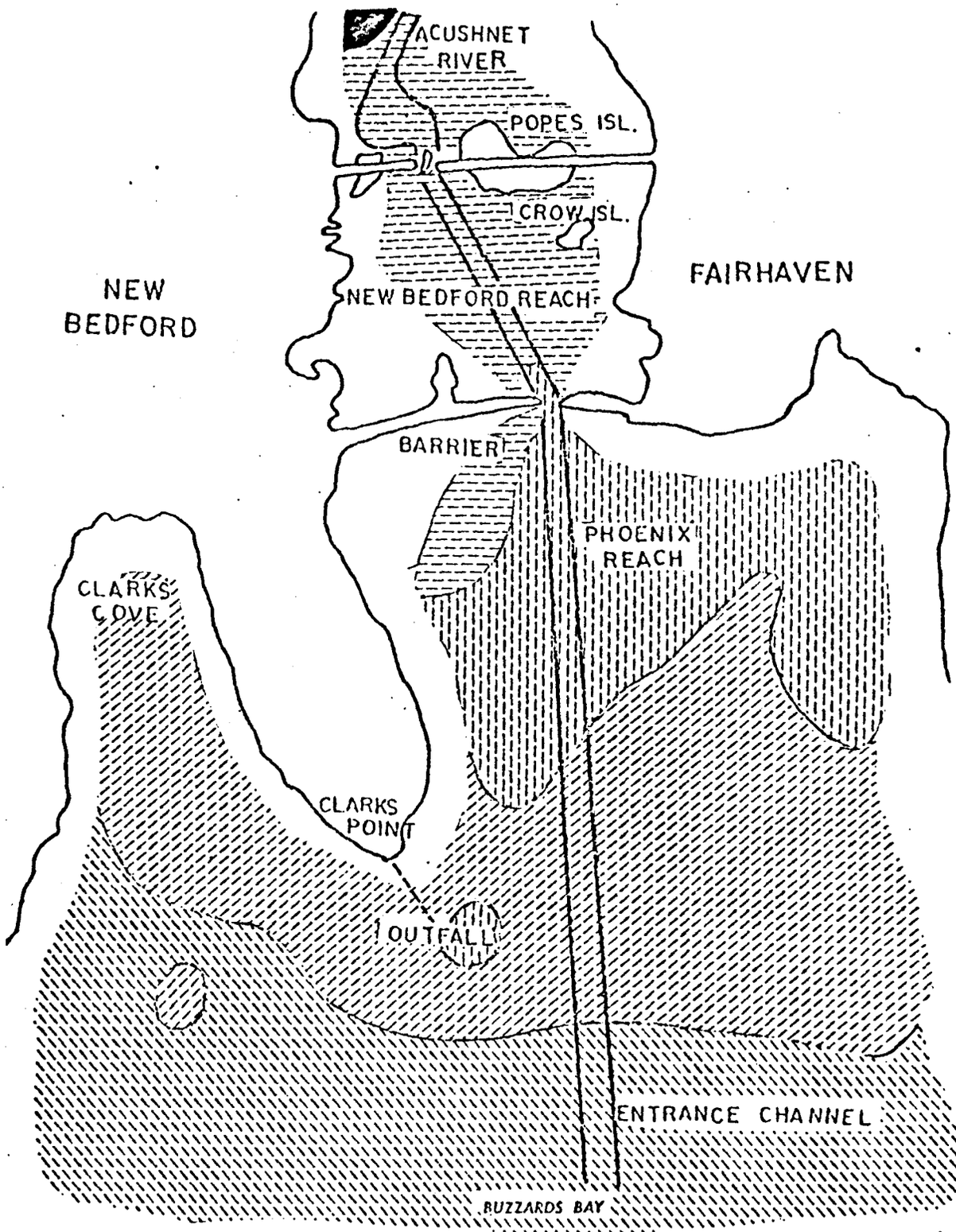
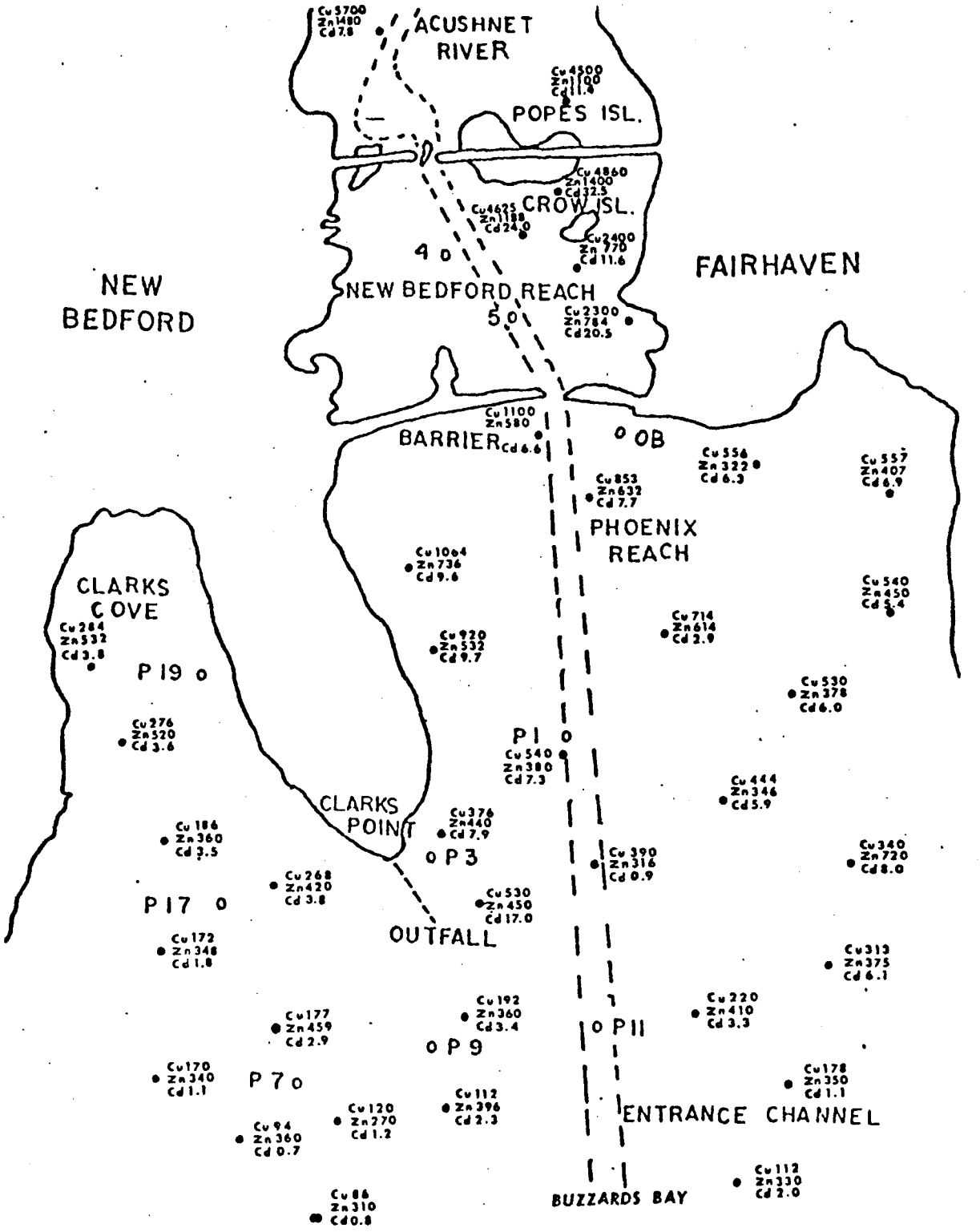

ACUSHNET RIVER
POPES ISL.
CROW ISL.
NEW BEDFORD
FAIRHAVEN
NEW BEDFORD REACH
BARRIER
PHOENIX REACH
CLARKS COVE
CLARKS POINT
OUTFALL
ENTRANCE CHANNEL
BUZZARDS BAY

Figure 2. Graphic summary of the sediment copper, zinc and cadmium concentrations (in ppm dry weight) of New Bedford Harbor. Dots represent points where sediment metal concentrations were determined. Metal data from Summerhayes et al. (1977). Empty circles represent stations sampled for macrobenthos.


ACUSHNET RIVER
POPES ISL.
CROW ISL.
NEW BEDFORD REACH
NEW BEDFORD
FAIRHAVEN
BARRIER
OB
PHOENIX REACH
CLARKS COVE
CLARKS POINT
OUTFALL
P19
P17
P7
P3
P1
P9
P11
ENTRANCE CHANNEL
BUZZARDS BAY
Cu 3700 Zn 1480 Cd 7.8
Cu 4500 Zn 1100 Cd 11.8
Cu 4860 Zn 1400 Cd 32.5
Cu 4625 Zn 1188 Cd 24.0
Cu 2400 Zn 770 Cd 11.6
Cu 2300 Zn 784 Cd 20.5
Cu 1100 Zn 580 Cd 6.6
Cu 556 Zn 322 Cd 6.3
Cu 557 Zn 407 Cd 6.9
Cu 853 Zn 632 Cd 7.7
Cu 1064 Zn 736 Cd 9.6
Cu 540 Zn 450 Cd 5.4
Cu 714 Zn 614 Cd 2.9
Cu 920 Zn 532 Cd 9.7
Cu 284 Zn 532 Cd 3.8
Cu 530 Zn 378 Cd 6.0
Cu 276 Zn 520 Cd 3.6
Cu 540 Zn 380 Cd 7.3
Cu 444 Zn 346 Cd 5.9
Cu 376 Zn 440 Cd 7.9
Cu 186 Zn 360 Cd 3.5
Cu 390 Zn 316 Cd 0.9
Cu 340 Zn 720 Cd 8.0
Cu 268 Zn 420 Cd 3.8
Cu 530 Zn 450 Cd 17.0
Cu 172 Zn 348 Cd 1.8
Cu 313 Zn 375 Cd 6.1
Cu 177 Zn 459 Cd 2.9
Cu 192 Zn 360 Cd 3.4
Cu 220 Zn 410 Cd 3.3
Cu 170 Zn 340 Cd 1.1
Cu 178 Zn 350 Cd 1.1
Cu 120 Zn 270 Cd 1.2
Cu 112 Zn 396 Cd 2.3
Cu 94 Zn 360 Cd 0.7
Cu 112 Zn 330 Cd 2.0
Cu 86 Zn 310 Cd 0.8

One of the objectives of this study was to gather baseline data on the metal concentrations of the macrobenthos of New Bedford Harbor. These metal levels will provide a set of baseline values for the area against which future studies of the harbor can be compared. A second objective was to test the use of C. fornicata as a coastal metal pollution monitor using data from New Bedford Harbor animals. The relationship between slipper limpet weight and tissue metal concentration was investigated. Animals from four locations, each with different estimated sediment metal concentrations, were compared to see if significant differences occurred among the group metal concentrations. The correlations between slipper limpet and estimated sediment metal concentrations were also studied to determine whether the species reflected the environmental metal levels.

MATERIALS AND METHODS

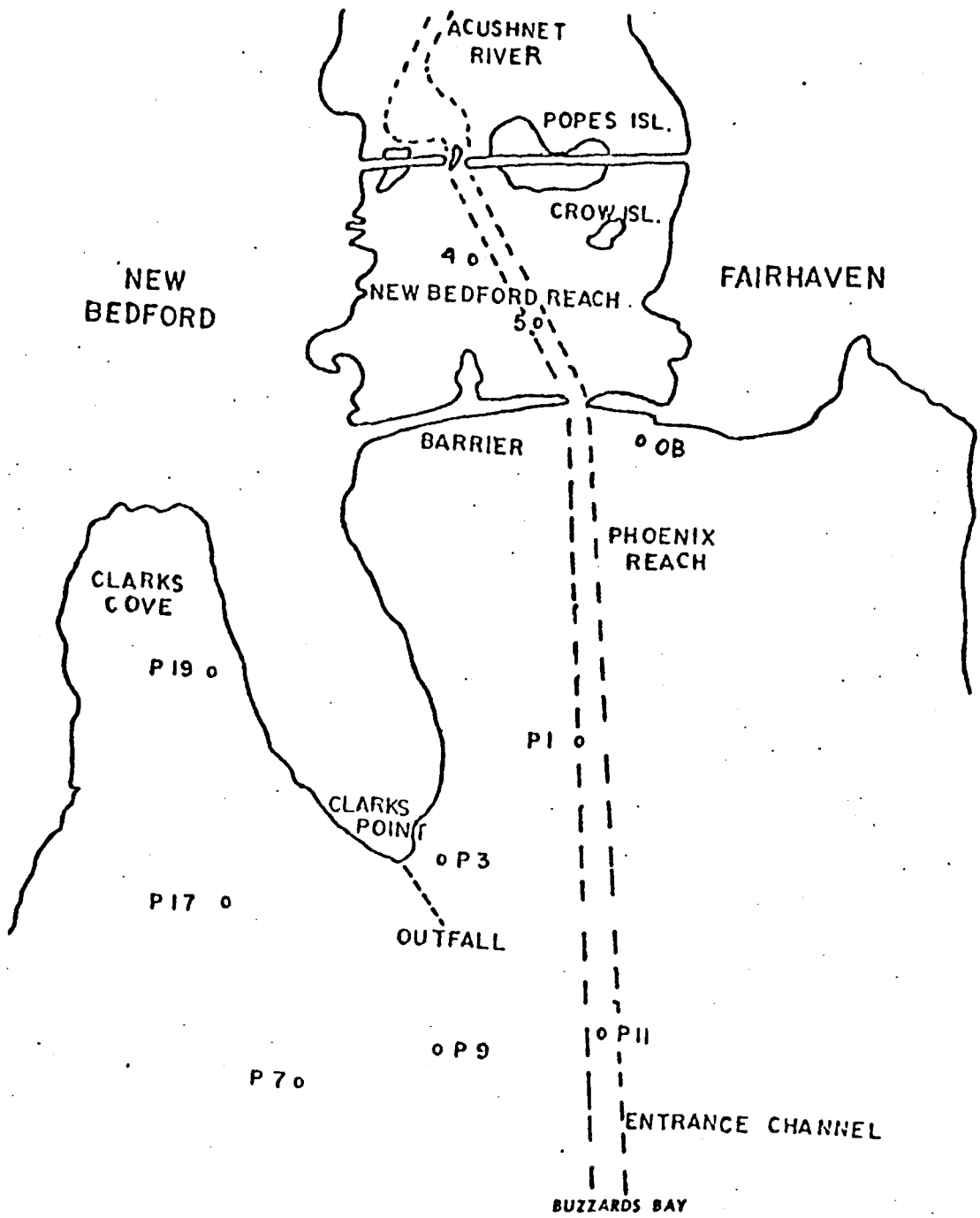
Cruise I-Quantitative Survey. An initial quantitative survey of the macrobenthos of the Harbor area was conducted aboard the R/V Asterias on November 25 and 26, 1975. Nine sampling stations were chosen, seven outside the harbor (P1, P3, P7, P9, P11, P17, P19) and two in the inner harbor (4, 5). Table 1 lists the coordinates of these stations, and Figure 3 portrays the station locations. Two grab samples were taken for organisms at each station with a 1/25 m^2 van Veen bottom grab. A third grab was taken but only a small subsample of sediment was kept and frozen for future analysis of carbon content. Samples were sieved on board ship, and animals retained on a 0.42 mm mesh screen were preserved in a 5% buffered solution of formalin in sea water. Animals were sorted in the laboratory; polychaetes were identified to family and molluscs to species, with no further identifications performed on oligochaetes or sipunculids. Individuals were counted under a dissecting scope, and a Folsum splitter was used when necessary to divide bountiful samples. Mollusc, 'worm' and total wet biomasses were obtained for each grab, the 'worm' biomass being the pooled wet weights for polychaetes, sipunculids and oligochaetes. Carbon analyses of the sediments were performed at Woods Hole Oceanographic Institution using a Leco Carbon/Nitrogen Analyzer.

Cruise II-Qualitative Survey. Four of the original nine sampling stations were revisited for a qualitative survey to test whether sufficient numbers of certain species could be

Table 1. Sampling station locations.

STATION NAME	LATITUDE (N)	LONGITUDE (W)
4	41° 38′ 02″	70° 54′ 58″
5	41° 37′ 38″	70° 54′ 30″
P1	41° 36′ 16″	70° 53′ 30″
P3	41° 35′ 29″	70° 53′ 51″
P7	41° 34′ 05″	70° 54′ 56″
P9	41° 34′ 17″	70° 53′ 50″
P11	41° 34′ 33″	70° 52′ 34″
P17	41° 35′ 10″	70° 55′ 01″
P19	41° 36′ 08″	70° 55′ 00″
OB	41° 37′ 23″	70° 54′ 12″

Figure 3. Map of sample station locations in the New Bedford Harbor area.


ACUSHNET
RIVER
POPES ISL.
CROW ISL.
4 o
NEW BEDFORD REACH
5 o
NEW
BEDFORD
FAIRHAVEN
BARRIER
o OB
PHOENIX
REACH
CLARKS
COVE
P 19 o
P1 o
CLARKS
POINT
o P3
P17 o
OUTFALL
o P11
o P9
P7 o
ENTRANCE CHANNEL
BUZZARDS BAY

obtained for metal analyses both by the methods used and at the stations chosen. Station 5 in the inner harbor and Stations P3, P17 and P19 outside the harbor were sampled in June and July of 1976 from the R/V Asterias. Animals were procured with a 0.1 m^2 van Veen bottom grab. A small subsample of sediment was taken from each station and frozen, to be analyzed later for carbon content. Samples were sieved, preserved and sorted as described in the preceeding section. Only the larger polychaetes were examined, and these were identified to species.

Cruise III-Collection of Organisms for Metal Analysis. A third cruise was undertaken in April, 1977, aboard the R/V Corsair to secure animals for metal analysis. Stations sampled were P9 and P17 off Clark's Point, Station 5 in the inner harbor, and a new location, OB, outside the harbor and just east of the barrier opening (Fig. 3). These sampling locations were chosen because each supported large populations of C. fornicata. In addition, the estimated environmental sediment metal concentrations varied between the stations, with Stations 5 and OB having the higher concentrations of copper, zinc and cadmium (Table 2). Therefore, the metal concentrations of conspecifics from stations with different sediment levels could be compared.

Animals were obtained by dredging with an epibenthic sled. Samples were inspected on deck, and living molluscs and crustaceans were washed with clean salt water and immediately frozen in clean plastic jars.

Table 2. Estimated sediment metal concentrations at sampling stations used in Cruise III. Sediment concentrations are approximated from known concentrations at adjacent stations (see Figure 2). Metal concentrations are in ppm dry weight. Sediment metal concentration data from Summerhayes *et al.* (1977).

STATION	Cu	Zn	Cd
5	2200	750	20.0
OB	900	600	6.5
P17	220	400	3.5
P9	160	360	3.0

Metals Determination. Molluscs were removed from their shells, metal analyses being done on the soft parts only. Several individuals were sometimes pooled to obtain sufficient material for analysis. For crabs, metal analyses were performed on the entire animal, including the carapace. Libinia (spider crabs) were heavily covered with algae and detritus. Most of this could be scraped off, but some residue remained on the carapace to be included in the metal analyses. The other crab species posed no such problem, having cleaner carapaces.

Methods for the preparation of tissue for metal analysis were basically those of Anderson (1972). Animals were thawed at room temperature and then rinsed with double distilled water (DDW). To obtain a dry weight, the sample was dried at 95-100°C for 48 hours or until a constant weight was attained. Samples were then placed in acid washed crucibles or glass beakers and combusted in a muffle furnace at 450-500°C for 16-20 hours. Tissues were dry ashed prior to analysis as the per cent recovery of metals in this technique is greater than that in wet digestion methods (Anderson, 1972).

One to four ml. of concentrated reagent grade nitric acid were added to the ashed samples, and sufficient time was allowed for complete digestion of the sample. Samples were then suction filtered through acid washed Whatman 0.45u mesh silicon filters to remove any undissolved particulates which might clog the aspirator tube of the Atomic Absorption Spectrophotometer (AAS). Each filtrate was transferred to a

volumetric flask and diluted to volume with DDW and nitric acid. The final volume of a sample varied since the concentration of the metal in the sample solution to be analyzed had to fall within the operating range of the Spectrophotometer for that metal. For the Perkin-Elmer Model 290 B AAS, these optimum operating ranges were Cu 2-20 ug/ml (ppm), Zn 0.2-3 ppm and Cd 0.5-5 ppm. However, each final volume was kept between 5 to 10% nitric acid in DDW. The concentrations of copper, zinc and cadmium in the diluted samples were measured by direct aspiration into the AAS unit. Final tissue concentrations were corrected for dilution and expressed as parts per million dry weight (ppm), or ug/g. Appropriate blanks were run with each sample batch.

Stock solutions of 1000 ppm Cu, 1000 ppm Cd and 500 ppm Zn were prepared from the respective pure metals as described in the Standard Methods Manual (Perkin-Elmer Co., 1968). Standards within the optimum operating range for each metal were prepared from the stock solutions to generate working calibration curves for each metal. Sample or standard absorption registering on the AAS was graphed on a Continuous Strip Chart Recorder. The height of each standard absorption peak was measured. Plots of standard metal concentrations in ppm versus corresponding heights of absorption peaks gave calibration curves, which were essentially linear for copper but slightly curvilinear for zinc (Figures 4 and 5). Therefore, an increased number of standard solutions were made in order to obtain more points for the calibration curves of both

Figure 4. Calibration curve for copper standards.

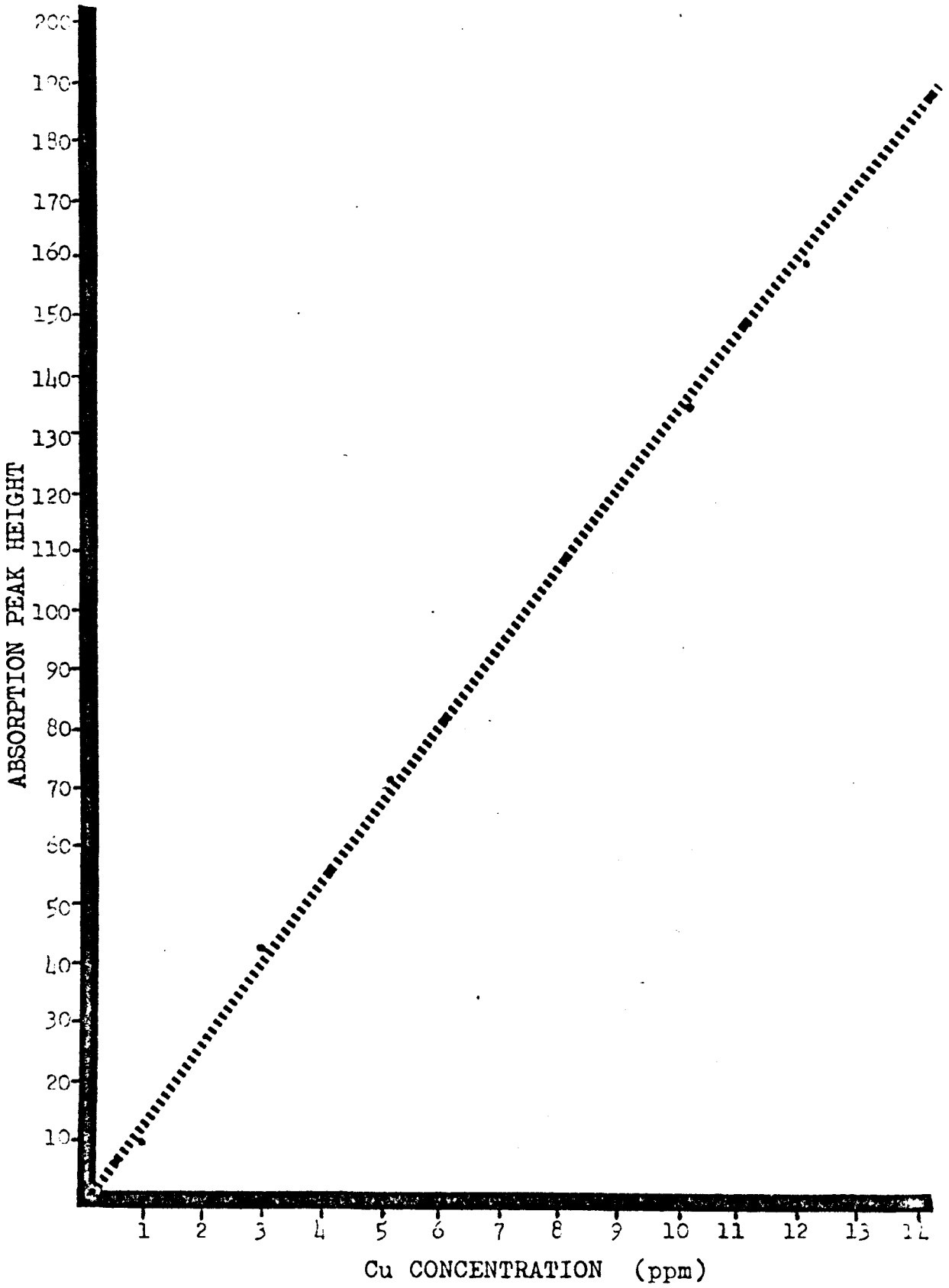
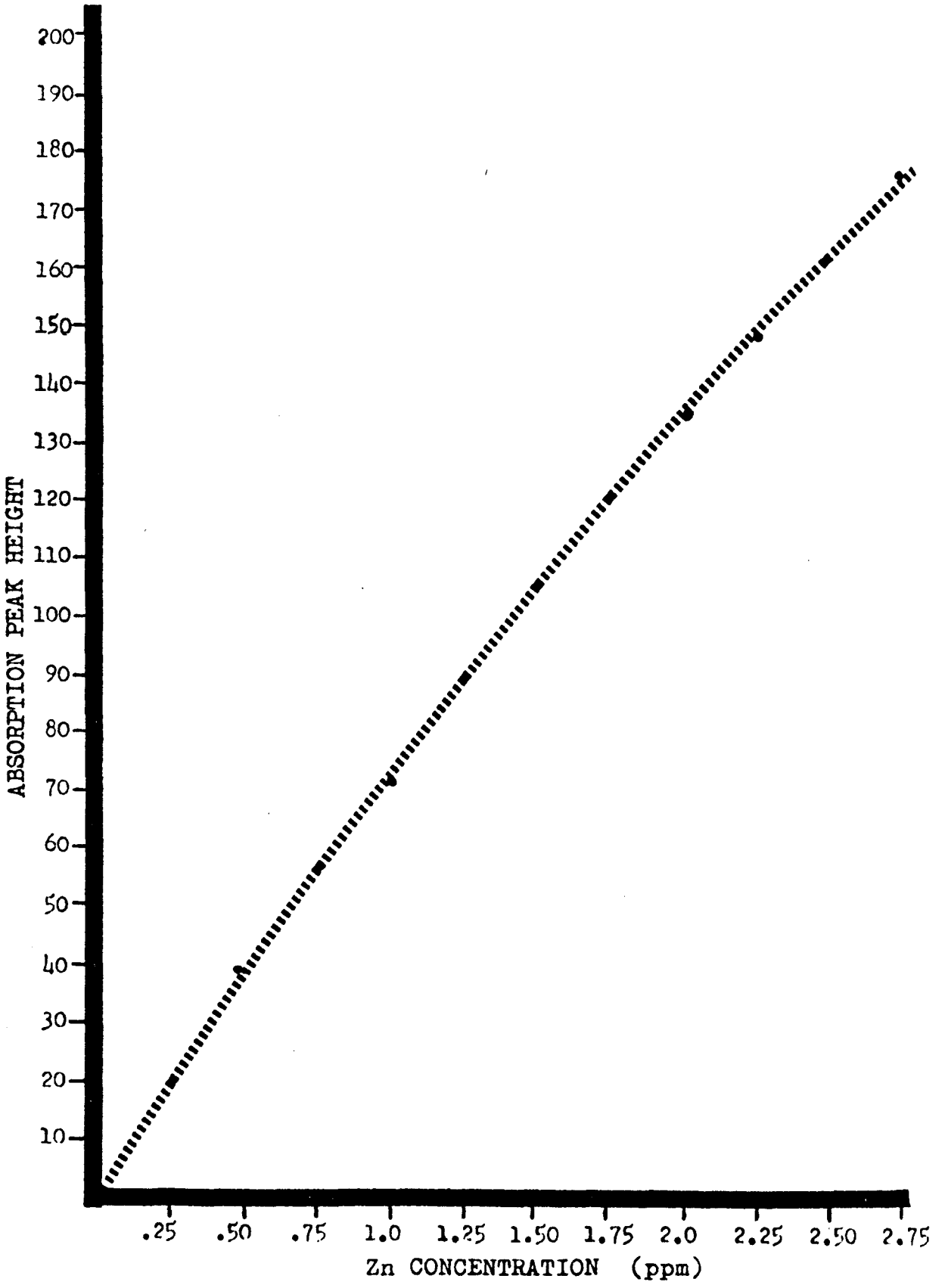

ABSORPTION PEAK HEIGHT
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
10
11
12
13
Cu CONCENTRATION (ppm)

Figure 5. Calibration curve for zinc standards.


ABSORPTION PEAK HEIGHT
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
.25
.50
.75
1.0
1.25
1.50
1.75
2.0
2.25
2.50
2.75
Zn CONCENTRATION (ppm)

elements. Diluted sample metal concentrations were obtained through use of these working curves which were generated for each group of samples. Final tissue concentrations in ug/g dry weight (ppm) were derived from the diluted sample concentrations by the equation:

ppm dry wt.=(ppm of diluted sample X dilution)/dry wt.

Cadmium analyses were done on a Perkin-Elmer 603 AAS at the Environmental Protection Agency Environmental Quality Laboratory in Narragansett, Rhode Island, after preliminary tests showed sample cadmium levels to be below the detection limit of the 290 B AAS. The same samples were analyzed for copper and zinc concentrations on both the Perkin-Elmer 290 B and 603 Spectrophotometers for comparative purposes. The Perkin-Elmer 603 has a deuterium lamp background corrector to minimize matrix interference and background absorption, and gives sample concentrations directly on a digital display. Comparison of the 290 B and 603 values showed good agreement in the data (Table 3), giving confidence to the 290 B values; differences between the two sets of data were not significant for Crepidula fornicata (Cu: $F=0.011$ N.S., d.f. 1,18 $p>0.75$; Zn: $F=2.45$ N.S., d.f. 1,18 $p=0.16$) or Mercenaria mercenaria samples. Both instruments were indicating similar concentrations.

The next step was a test of the technique of metal recovery from the tissue itself. The EPA supplied a standard clam homogenate Mercenaria mercenaria of 'known' quality, the metal concentrations having been established on samples prepared

Table 3. Comparison of the sample Zinc and Copper concentrations determined by two Atomic Absorption Spectrophotometers. Diluted sample concentrations were corrected for dilution, giving the actual concentration of the sample in ppm dry weight.

(DILUTED) COPPER		(CORRECTED) COPPER		(DILUTED) ZINC		(CORRECTED) ZINC	
290 B	603	290 B	603	290 B	603	290 B	603
			Crepidula fornicata				
8.2	8.2	95.0	95.0	6.8	7.4	78.8	85.7
3.3	3.2	88.3	85.6	3.3	3.6	88.3	96.3
2.9	2.8	65.6	63.4	4.0	4.4	90.5	99.5
4.5	4.4	96.4	94.3	3.3	3.4	70.7	72.8
6.2	5.9	133.8	127.3	3.7	4.0	79.8	86.2
10.8	10.3	271.9	259.3	4.0	4.4	100.7	110.7
5.2	5.3	129.3	131.8	3.8	4.1	94.5	101.9
3.6	3.6	129.1	129.1	2.5	2.8	89.6	100.2
7.0	7.0	153.7	153.7	3.8	4.0	83.4	87.8
5.7	5.6	170.2	167.3	2.5	2.8	77.7	83.6
			Mercenaria mercenaria				
1.7	1.6	79.6	74.9	5.6	5.6	262.3	262.3
1.9	1.8	60.9	57.7	9.3	9.4	297.9	301.1

by wet digestion techniques, which differ from the dry ashing method used in this study. Comparison of the dry ashing values and the EPA's accepted values for this homogenate (Table 4) showed my copper concentration to be significantly greater than the accepted copper concentration ($t=32.5^*$ d.f. 1, $p<0.05$). No significant differences were found between zinc ($t=4.33$ N.S., d.f. 1, $p=0.17$) or cadmium ($t=7.69$ N.S., d.f. 1, $p=0.09$). Although the difference in copper concentrations was significant, such disparity in metal concentrations between different laboratories is commonplace (Capar, 1977), and such values as determined by the dry ashing technique here can be considered valid (personal communication, Walter Galloway, Director of Atomic Absorption Spectrophotometry, EPA, Narragansett). It is believed that some slight copper contamination introduced during sample preparation was responsible for the higher homogenate copper concentration, rather than the efficiency of metal recovery by the method used. However, since Atomic Absorption analysis involves measuring metal concentrations at extremely low levels, the slightest discrepancy will become significant.

Table 4. Comparison of the recovery of metals from standard clam homogenate by the dry ashing method versus known EPA concentrations. All concentrations are in ppm dry weight. t-test comparing mean metal concentrations of sample prepared by dry ashing method with accepted metal concentrations of the standard.

		CONCENTRATIONS		
		Cu	Zn	Cd
(Samples prepared by dry ashing technique)	#1	28.7	139.7	1.64
	#2	26.2	145.5	1.57
Dry ashing average (± one std. dev.)		27.4±1.8	142.6±4.1	1.60±0.05
EPA concentrations (± one std. dev.)		23.5±0.8	144.0±5.0	1.50±0.10

Comparisons of copper concentrations, $t = 32.50^{*}$ $p < 0.05$

Comparison of zinc concentrations, $t = 4.33$ N.S.

Comparison of cadmium concentrations, $t = 7.69$ N.S.

RESULTS

Cruise I-Quantitative Survey. Table 5 summarizes the results of the initial quantitative faunal survey, while Table 6 lists wet weight data and organic carbon content of the sediments at the stations. Molluscs were weighed with their shells. Comparison of mollusc biomass among stations gave a relative idea of where molluscs were more predominant.

Stations P9, P17 and 5 were the locations with the greatest molluscan wet weights. Epifaunal molluscs, namely Crepidula fornicata and C. plana, were the dominant organisms by weight at these stations. The deposit feeding bivalves Nucula proxima and Yoldia limatula were common at Stations P3 and P19. It was decided to resample at Stations P17 and 5 to ascertain the relative abundances of C. fornicata there, and to do similarly at Stations P3 and P19 for N. proxima and Y. limatula. These organisms appeared to be common in the study area, although mutually exclusive, and were considered as possible metal pollution monitors.

Cruise II-Qualitative Survey. Results of the qualitative survey are shown in Table 7. From this data, it became apparent that sufficient quantities of neither N. proxima nor Y. limatula could be recovered for metal analysis. Initial results with the 290 B AAS indicated that due to the sensitivity of the instrument, an organism substantially larger than N. proxima had to be used. Yoldia limatula was not common enough, as this survey confirmed. Crepidula fornicata was consistently common at Stations P17 and 5, and was of

Table 5. Faunal list of organisms recovered on Cruise I - quantitative sampling.

	STATIONS															
	4	5A	5B	P1	P3A	P3B	P7A	P7B	P9A	P9B	P11A	P11B	P17A	P17B	P19A	P19B
POLYCHAETES																
Ampharetidae							2									
Capitellidae		*	100	24	2128	4688	768	1440	436	76	1152	*	252	776	*	150
Cirratulidae						8		38	206	10	2	2	26	42	1	10
Dorvillidae								2		6			16			
Glyceridae	2		2		28	20		14	8			4	4	4	10	16
Hessionidae									2							
Lumbrineridae							30	68				3				
Nereidae	3			1								1				
Nephtyidae						4		4		1	14	9			1	2
Orbiniidae			2			48				1			2	4	20	34
Paraonidae					20		26	40	102	46		3		14		
Phyllodocidae		20	20			64	4			6			4			
Spionidae	267	3	14		16	80	6	8	4		48	87	8	6	2	
Syllidae		1	8	4		48	22	152	112	80	38	24		10		8
Terebellidae									2							
Trichobranchidae									2							
GASTROPODS																
Acteon punctostriatus				1		6										
Crepidula convexa																2
Crepidula fornicata	18	4	14						40	12			28	39		
Crepidula plana	22		22										6	20	1	
Haminoea solitaria						8						1				16
Mitrella lunata						2	2	4	8		1	1				
Nactica pusilla					4	2	2	4	4		2					
Nassarius trivattatus				1	4											2
Retusa sp.				1		4					3	2			2	
BIVALVES																
Anomia simplex			2							5						
Cardita borealis							1	20								
M. mercenaria		1			1				44							
Nucula proxima				4	16	26			4		5	8			9	12
Pandora gouldiana														2		
Yoldia limatula				1		2					1				5	12
SIPUNCULIDS					13	20	14	30	8		4		3		1	6
OLIGOCHAETES					64	60	2	6	56		8		2		1	18

* loss of an untold number of Capitellids (accidentally discarded)

Table 6. Animal biomasses and sediment % organic carbon for stations sampled quantitatively. Biomasses in grams wet weight.

STATION	TOTAL BIOMASS	'WORM' BIOMASS	MOLLUSC BIOMASS	TOTAL NUMBER OF INDIVIDUALS	% ORGANIC CARBON IN SEDIMENTS
4 A	3.9441	0.6670	3.2771	322	4.5
5 A*	13.1155	0.0110	13.1045	29	-
5 B	17.2644	0.0462	17.2182	208	-
P1 A	0.3234	0.2418	0.0816	41	-
P3 A	4.6956	0.2296	4.4660	2293	-
P3 B	1.6729	0.8628	0.8101	5090	-
P7 A	0.0806	0.0566	0.0240	879	0.7
P7 B	0.4482	0.2402	0.2080	1830	same
P9 A	19.8320	0.1964	19.6356	1054	-
P9 B	26.5183	0.0198	26.4985	251	-
P11 A	0.3768	0.2566	0.1202	1279	1.4
P11 B*	0.3364	0.3147	0.0217	145	same
P17 A	13.4017	0.0404	13.3613	367	1.7
P17 B	19.2492	0.0740	19.1752	945	same
P19 A*	0.3610	0.0859	0.2751	53	1.5
P19 B	0.7840	0.2158	0.5682	292	same

* loss of an untold number of Capitellids (accidentally discarded)

Table 7. Results of Cruise II - qualitative sampling.

	STATION P17			STATION 5		STATION P19			STATION P3			
	Grab 1	Grab 2	Grab 3	Grab 1	Grab 2	Grab 1	Grab 2	Grab 3	Grab 1	Grab 2	Grab 3	Grab 4
POLYCHAETES												
Glycera americana									1			
Lumbrineris acuta				1								
Nephtys incisa									4	8	3	4
Nephtys sp.									1			
Pherusa affinis										1		
NEMERTEANS												
Cerebratulus lacteus										1		
GASTROPODS												
Anarchis translirata					1							
Nassarius trivattatus								2	1			
Crepidula fornicata	25		15	35	35	10	2	3				
Crepidula plana	4		2	20	7	2						
BIVALVES												
Anadara transversa			1									
Anomia simplex	1		1			1						
Ensis directus				1								
Mercenaria mercenaria	1	2	1	2	2	2		2				
Mulinia lateralis										1		
Nucula proxima									50	150	40	15
Pandora gouldiana		1	3									
Tellina agilis				1	1		3					
Yoldia limatula								1	4		3	10
DECAPODS												
Neopanope texana		2		6	2							
Pagarus longicarpus		1										

sufficient size for metal analysis. However, a more efficient means of sampling was desired, one which would also enable the capture of some of the more mobile epifaunal species. An epibenthic sled was found to satisfy these requirements.

Cruise III-Organisms Collected For Metal Analysis. Metal concentrations of molluscs and crustaceans sampled by the epibenthic sled are summarized in Tables 8, 15 and 17.

Table 8 lists the copper, zinc and cadmium concentrations for samples of the slipper limpet C. fornicata. Metal concentrations were transformed to a logarithmic scale to achieve homogeneity of variances (Sokal and Rohlf, 1969). The relationships between slipper limpet weight and tissue metal concentration were investigated by correlation analysis. Average dry weight data were available for samples from three of the four stations. These average dry weights were also transformed to logarithms, as previous investigators have demonstrated this transformation to be applicable in weight/metal concentration correlations (Boyden, 1974; Phillips, 1976b). However, to avoid negative characteristics in the logarithms, cadmium concentrations and the average dry weights (both of which included numbers between zero and one) were first coded by adding 1 to all variates, and were then log transformed (Sokal and Rohlf, 1969). Zinc and copper concentrations were not coded before log transformation.

Table 8. Metal concentrations of Crepidula fornicata in ppm dry weight.

DRY WT. (g)	CONCENTRATIONS Cu	Zn	Cd	NUMBER OF INDIVIDUALS	AVERAGE DRY WT. (g)
		STATION 5			
0.286	74.3	120.6	4.37	1	0.286
0.212	254.1	172.6	13.00	1	0.212
0.537	242.2	128.1	3.26	5	0.107
0.619	-	156.7	4.04	3	0.206
0.364	237.0	120.2	4.12	2	0.182
0.334	228.5	119.1	5.99	4	0.084
0.303	132.0	124.6	4.95	2	0.152
0.246	325.9	99.8	5.09	2	0.123
0.443	146.7	94.8	3.39	4	0.111
0.587	189.5	99.7	3.41	3	0.196
0.388	164.5	134.2	5.16	32	0.012
0.419	265.7	130.2	2.98	8	0.052
0.386	187.8	121.8	4.53	8	0.048
0.372	144.6	117.7	4.71	15	0.025
2.418	165.4	105.9	3.93	-	-
3.444	139.4	92.9	3.78	-	-
1.748	185.9	94.4	3.15	-	-
2.060	145.6	99.5	3.40	-	-
1.986	271.9	100.7	2.77	-	-
		STATION P9			
0.322	178.3	117.8	2.33	2	0.161
1.428	147.1	84.0	1.40	3	0.476
0.588	352.9	91.0	2.13	1	0.588
0.818	111.5	95.3	1.83	15	0.055
0.383	117.5	86.2	1.96	2	0.192
0.340	77.1	67.6	1.47	1	0.340
1.458	178.3	93.9	1.71	-	-
2.814	155.5	79.9	1.42	-	-
0.486	61.7	84.3	2.06	-	-
1.909	115.2	90.6	1.57	-	-
1.690	130.2	89.9	1.78	-	-
1.741	201.0	95.4	2.30	-	-
4.316	95.0	78.8	1.40	-	-
2.235	93.9	87.2	-	-	-
2.209	65.6	90.5	0.90	-	-
2.334	96.4	70.7	1.10	-	-
2.318	133.8	79.8	0.86	-	-

Table 8. (cont'd) Metal concentrations of *Crepidula fornicata* in ppm dry weight.

DRY WT. (g)	CONCENTRATIONS Cu	Zn	Cd	NUMBER OF INDIVIDUALS	AVERAGE DRY WT. (g)
		STATION P17			
0.669	140.1	89.7	2.62	5	0.134
0.716	137.9	71.9	2.44	3	0.239
0.383	104.4	70.5	1.96	2	0.192
0.504	263.0	103.2	3.47	3	0.168
0.516	222.6	103.1	2.90	4	0.129
0.319	195.9	100.3	2.35	1	0.319
0.338	306.5	99.0	4.43	1	0.338
0.510	183.6	121.4	2.94	3	0.170
0.578	175.2	101.6	3.46	5	0.116
0.427	137.6	119.4	2.93	2	0.214
0.492	129.4	115.2	3.05	1	0.492
0.444	306.9	105.9	3.94	1	0.444
0.304	127.4	123.3	3.29	3	0.101
0.634	78.8	97.7	2.36	2	0.317
0.592	268.2	102.6	4.22	2	0.296
0.567	216.0	109.8	4.41	2	0.284
0.608	137.6	81.4	2.46	2	0.304
0.774	148.6	87.2	3.23	3	0.258
0.488	251.0	86.6	2.56	2	0.244
0.669	199.9	99.4	2.99	3	0.223
0.534	198.9	116.9	2.81	3	0.178
0.558	199.6	112.1	1.79	-	-
0.675	196.3	90.4	2.59	-	-
0.671	165.8	111.8	2.61	-	-
1.699	153.1	78.9	-	-	-
2.007	130.0	98.0	-	-	-
2.732	197.7	87.1	-	-	-
2.035	176.9	94.3	-	-	-
2.278	153.7	82.3	2.19	-	-
1.674	170.2	77.7	1.19	-	-
2.028	128.2	91.2	2.47	-	-
		STATION OB			
1.870	88.3	88.3	2.70	-	-
2.078	144.4	93.9	-	-	-
2.011	129.3	93.2	2.98	-	-
1.394	129.1	89.6	2.15	-	-

- signifies that either the metal listed or the number of individuals was not determined for that sample.

Nonsignificant correlations of log average dry weight versus log animal metal concentration were found for each element at each of the three stations (Table 9). Scattergrams of these two variables for each station are shown in Figures 6, 7 and 8. As there were no significant weight/concentration correlations for copper, zinc or cadmium in the slipper limpet, it was possible to compare the metal concentrations of animals of unlike size.

Correlations were performed of log estimated sediment metal concentrations versus log animal metal concentrations for each metal to test the degree to which the slipper limpet reflected the environmental sediment metal levels (Table 10). All sample concentrations were used in these correlations, as weight was found not to have any significant relationship with tissue metal concentrations. Significant correlations between animal and sediment metal concentrations existed for zinc ($r=0.529^{**}$ d.f. 69, $p<0.01$) and cadmium ($r=0.651^{**}$ d.f. 63, $p<0.01$), while copper exhibited no correlation ($r=0.211$ N.S., d.f. 68). Inspection of the mean animal metal concentrations for each station (Table 11) revealed Station P17's means to be much higher than expected relative to the estimated sediment metal concentrations for that station. A second correlation was performed of animal versus sediment metal concentrations, but this time without data from Station P17 (Table 10). The correlation for copper was now significant ($r=0.434^{**}$ d.f. 37, $p<0.01$), and those for zinc ($r=0.672^{**}$ d.f. 38, $p<0.01$) and for cadmium ($r=0.825^{**}$

Table 9. Correlations of log animal metal concentrations and log average dry weights for *Crepidula fornicata*.

STATION	d.f.	Cu	d.f.	Zn	d.f.	Cd
5	11	-.275 N.S.	12	.141 N.S.	12	.171 N.S.
P17	19	.117 N.S.	19	-.386 N.S.	19	.196 N.S.
P9	4	.542 N.S.	4	-.022 N.S.	4	-.265 N.S.

N.S. means that no significant correlation exists at the 95% level of confidence.

Figure 6. Plot of log metal concentration versus log average dry weight for Crepidula fornicata from Station P17.

Copper •

Zinc ▲

Cadmium ●

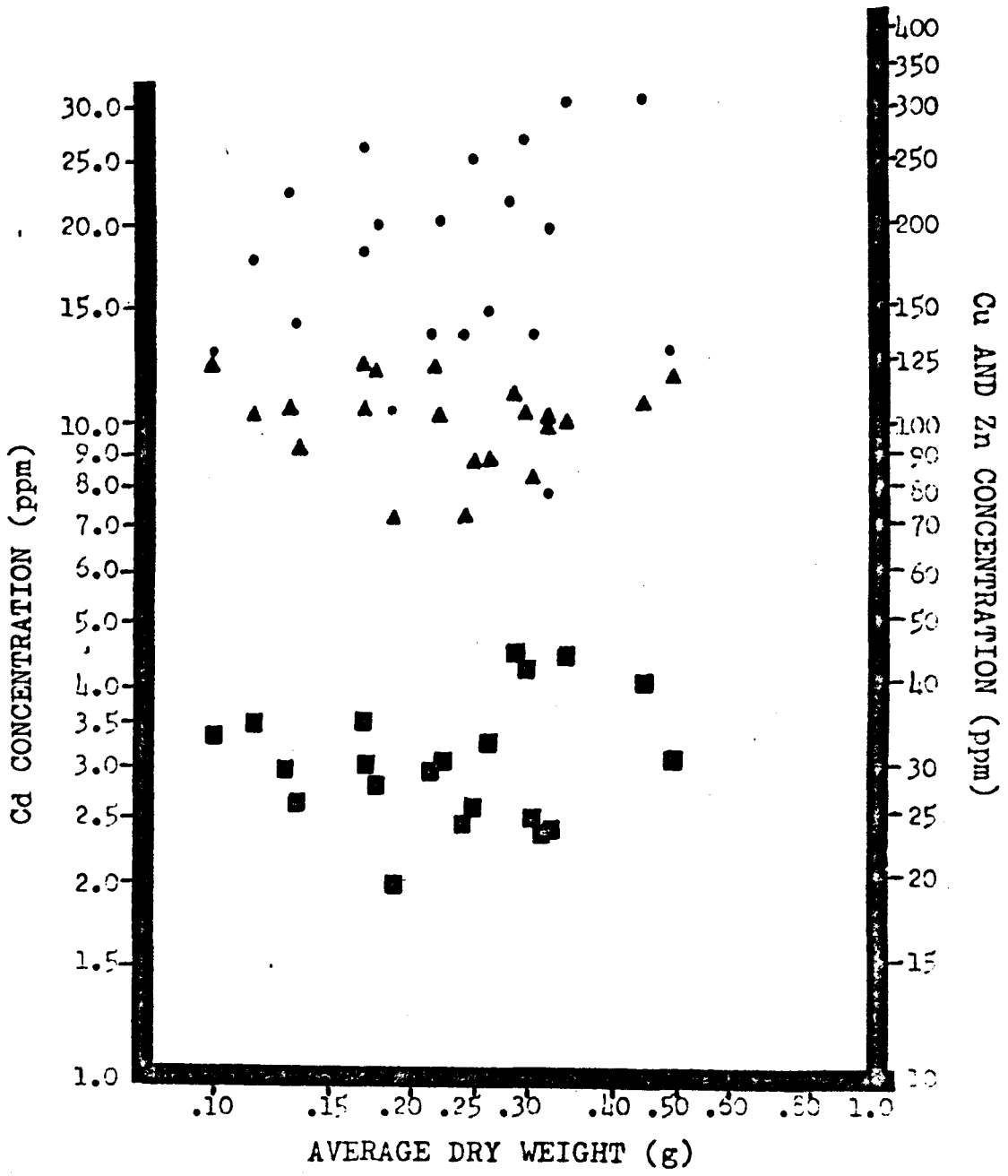

Cd CONCENTRATION (ppm)
30.0
25.0
20.0
15.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.5
3.0
2.5
2.0
1.5
1.0
.10
.15
.20
.25
.30
.40
.50
.60
.80
1.0
AVERAGE DRY WEIGHT (g)
Cu AND Zn CONCENTRATION (ppm)
400
350
300
250
200
150
125
100
90
80
70
60
50
40
30
25
20
15
10

Figure 7. Plot of log metal concentration versus log average dry weight for Crepidula fornicata from Station 5.

Copper ●

Zinc ▲

Cadmium ■

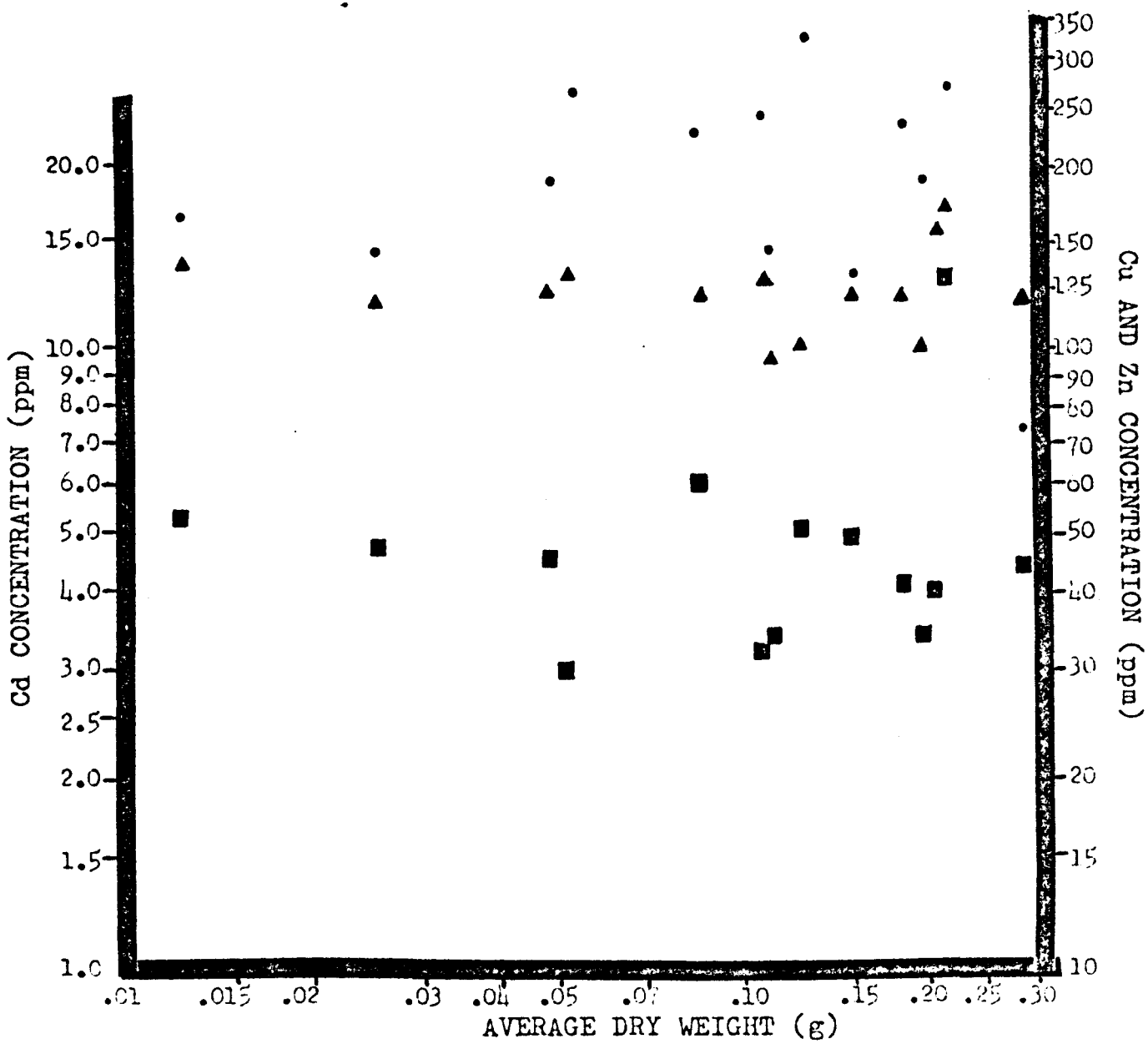

Cd CONCENTRATION (ppm)
20.0
15.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.5
2.0
1.5
1.0
Cu AND Zn CONCENTRATION (ppm)
350
300
250
200
150
125
100
90
80
70
60
50
40
30
20
15
10
.01
.015
.02
.03
.04
.05
.07
.10
.15
.20
.25
.30
AVERAGE DRY WEIGHT (g)

Figure 8. Plot of log metal concentration versus log average dry weight for Crepidula fornicata from Station P9.

Copper •

Zinc ▲

Cadmium ■

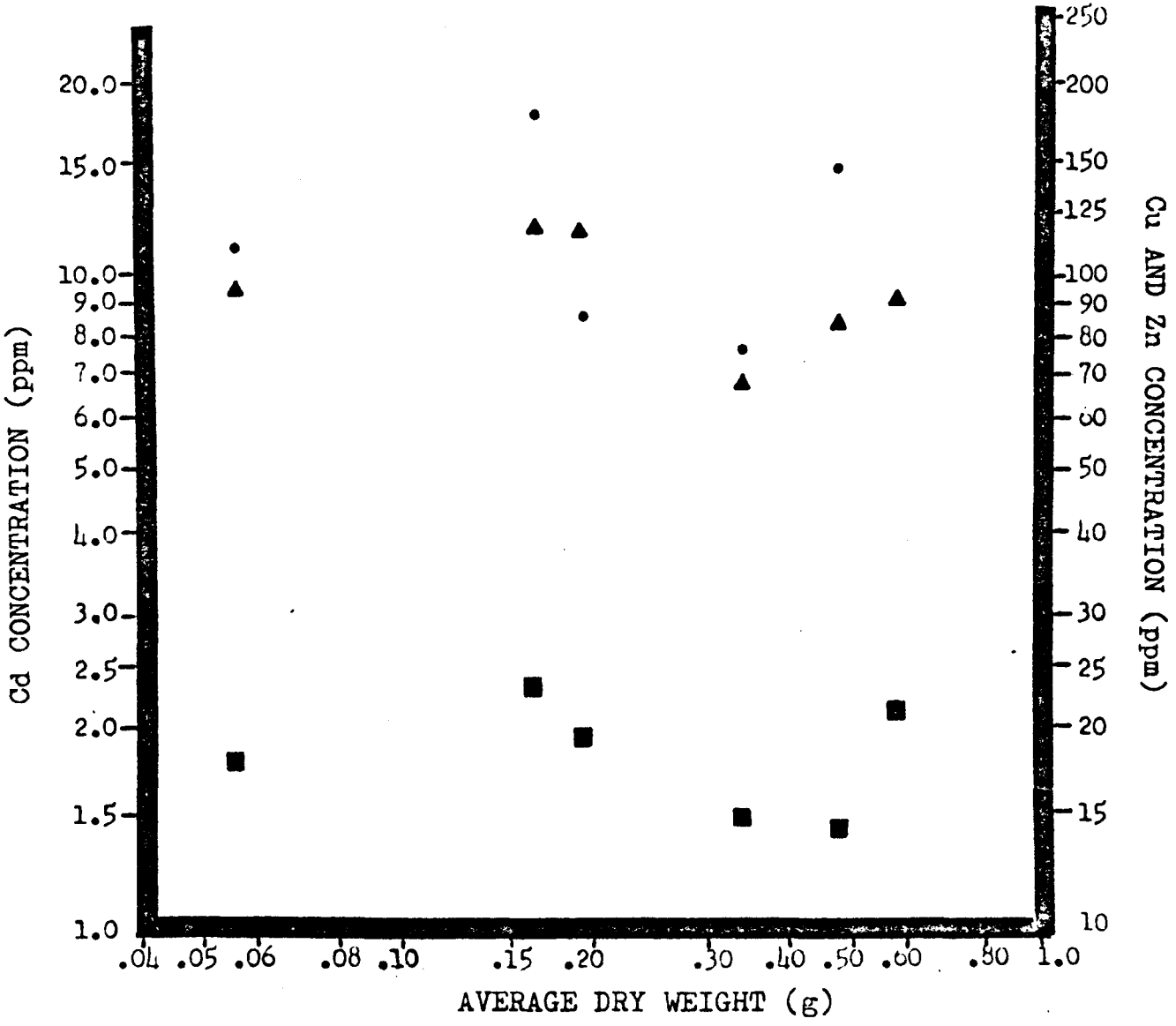

Cd CONCENTRATION (ppm)
20.0
15.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.5
2.0
1.5
1.0
Cu AND Zn CONCENTRATION (ppm)
250
200
150
125
100
90
80
70
60
50
40
30
25
20
15
10
.04 .05 .06 .08 .10 .15 .20 .30 .40 .50 .60 .80 1.0
AVERAGE DRY WEIGHT (g)

Table 10. Correlations of log animal metal concentrations and log sediment metal concentrations for *Crepidula fornicata*.

Cu		Zn		Cd	
d.f.	r	d.f.	r	d.f.	r
		All Stations			
68	.211 N.S.	69	.529**	63	.651**
		Without Station P17 Data			
37	.434**	38	.672**	36	.825**

* significant correlation at the 95% level of confidence

** significant correlation at the 99% level of confidence

Table 11. Station mean metal concentrations and 95% confidence limits for Crepidula fornicata. Mean slipper limpet metal concentrations are arranged in decreasing order. Corresponding estimated sediment metal concentrations are also listed. All concentrations are in ppm dry weight.

STATION	ESTIMATED SEDIMENT METAL CONCENTRATION	N	MEAN TISSUE METAL CONCENTRATION	L_1	L_2
		Cu			
5	2200	18	184.1	154.7	219.0
P17	220	31	172.6	154.1	193.3
P9	160	17	123.6	98.7	154.7
OB	900	4	120.8	85.3	170.9
		Zn			
5	750	19	115.9	106.6	125.9
P17	400	31	96.6	91.6	101.9
OB	600	4	91.2	86.9	95.7
P9	360	17	86.7	81.3	92.5
		Cd			
5	20.0	19	4.26	3.57	5.05
P17	3.5	27	2.80	2.50	3.12
OB	6.5	3	2.59	1.62	3.91
P9	3.0	16	1.60	1.36	1.86

d.f. 36, $p<0.01$) were greater than before. This procedure points out that metal concentrations of slipper limpets from Station P17 do not conform to a general trend of decreasing tissue metal concentrations with decreasing estimated sediment metal concentrations.

An analysis of variance was performed on the data for each metal to establish whether significant differences in slipper limpet metal concentrations existed among the stations. For a priori tests for all metals, the mean metal concentration of limpets from Stations 5 and OB was compared with that from P17 and P9, as these first two stations had higher sediment concentrations than the latter two. In addition, differences in the tissue concentrations between Stations 5 versus OB and between Stations P17 and P9 were investigated. The latter pair of stations had similar estimated sediment metal levels; therefore, animals at these stations would be expected to have similar metal concentrations.

For copper (Table 12), there was a highly significant added component due to treatment effects among stations ($F=5.42^{**}$ d.f. 3, 66 $p<0.01$). The difference between Stations 5 and OB versus Stations P17 and P9 was not significant ($F=1.39$ N.S., d.f. 1, 66 $p<0.25$). However, the copper concentrations of Station 5 animals were significantly greater than those from Station OB ($F=4.78^{*}$ d.f. 1, 66 $p<0.05$), as were those of Station P17 versus Station P9 ($F=10.09^{**}$ d.f. 1, 66 $p<0.01$). Crepidula fornicata from Station P17 had higher copper concentrations than Station OB animals, despite

Table 12. Analysis of variance of the mean copper concentrations of *Crepidula fornicata* from different stations.

SOURCE OF VARIATION	d.f.	SS	MS	F
Among Stations	3	0.374	0.125	5.42**
5 and OB vs. P17 and P9	1	0.032	0.032	1.39 N.S.
5 vs. OB	1	0.110	0.110	4.78*
P17 vs. P9	1	0.232	0.232	10.09**
Within Stations	66	1.533	0.023	

estimated sediment copper concentrations being four times lower at Station P17 (Table 11). Station 5 animals had the highest copper concentrations, coincident with the greatest sediment copper levels.

There was a highly significant difference among the station animal zinc concentrations (Table 13) ($F=12.37^{***}$ d.f. 3, 67 $p<0.001$). The mean of Stations 5 and OB was significantly greater than that of Stations P17 and P9 ($F=23.36^{***}$ d.f. 1, 67 $p<0.001$). The zinc concentrations of slipper limpets from Station 5 were significantly greater than those from Station OB ($F=8.94^{**}$ d.f. 1, 67 $p<0.01$), and those of individuals from Station P17 were significantly greater than those from Station P9 ($F=6.28^{*}$ d.f. 1, 67 $p<0.05$). Estimated sediment zinc concentrations were lower at Station P17 than at OB, yet P17 animals had a higher mean zinc concentration than those at Station OB (Table 11). Station 5 again had slipper limpets with the highest tissue concentration of a metal (zinc) coincident with the greatest sediment metal concentration. Station P9 individuals and sediment had the lowest zinc concentrations of the four stations.

For cadmium (Table 14), there was a highly significant difference among the station mean slipper limpet metal concentrations ($F=27.20^{***}$ d.f. 3, 61 $p<0.001$). All three a priori tests were significant, with the mean of Stations 5 and OB significantly greater than that of Stations P9 and P17 ($F=47.20^{***}$ d.f. 1, 61 $p<0.001$), Station 5 significantly

Table 13. Analysis of variance of the mean zinc concentrations of Crepidula fornicata from different stations.

SOURCE OF VARIATION	d.f.	SS	MS	F
Among Stations	3	0.154	0.051	12.37***
5 and OB vs. P17 and P9	1	0.093	0.093	23.36***
5 vs. OB	1	0.036	0.036	8.94**
P17 vs. P9	1	0.025	0.025	6.28*
Within Stations	67	0.279	0.004	

Table 14. Analysis of variance of the mean cadmium concentrations of Crepidula fornicata from different stations.

SOURCE OF VARIATION	d.f.	SS	MS	F
Among Stations	3	0.817	0.272	27.20***
5 and OB vs. P17 and P9	1	0.472	0.472	47.20***
5 vs. OB	1	0.072	0.072	7.20**
P17 vs. P9	1	0.273	0.273	27.30***
Within Stations	61	0.585	0.010	

greater than Station OB ($F=7.20^{**}$ d.f. 1, 61 $p<0.01$), and Station P17 significantly greater than Station P9 ($F=27.30^{***}$ d.f. 1, 61 $p<0.001$). Station P17 individuals had greater cadmium concentrations than those from Station OB, despite sediments at Station OB having twice the estimated cadmium concentration of Station P17 sediments (Table 11). Station 5 animals and sediments again had the greatest cadmium concentrations, as in the case of zinc and copper, and Station P9 slipper limpets and sediments had the lowest cadmium concentrations, as with zinc.

For all three metals, therefore, slipper limpets at Station P17 showed rather aberrant metal concentrations relative to the estimated sediment metal concentrations. This might have been due to local high metal concentrations in the sediments of the area by Station P17. For zinc and cadmium, however, the overall correlations between estimated sediment metal concentrations and slipper limpet metal concentrations were highly significant, suggesting the credibility of C. fornicata as a pollution monitor for these metals. In addition, the ANOVAS for these metals showed that the mean metal concentration of Station 5 and OB slipper limpets (from locations with greater estimated sediment metal concentrations) was significantly greater than the mean of animals from Stations P17 and P9. Crepidula fornicata showed no significant correlation of its copper concentration with estimated sediment copper concentrations. The ANOVA of slipper limpet copper concentrations revealed the mean copper concentration

of individuals from Stations 5 and OB not to be significantly different than that of conspecifics from Station P17 and P9. Crepidula fornicata's use for monitoring copper pollution is therefore doubtful. Phillips (1976a,b) considered using Mytilus edulis as a monitor for several metals, with the exception of copper. The uptake of copper by the mussel was erratic and unpredictable. Such a situation may be applicable to C. fornicata.

Leatherland and Burton (1974) reported values of 116 ppm zinc and 0.7 - 1.4 ppm cadmium for C. fornicata from the Solent, England. These cadmium values are lower than those of New Bedford Harbor conspecifics, while the zinc concentrations are similar. From a different part of the Solent, slipper limpet concentrations of 270 ppm copper, 940 ppm zinc and 3.9 ppm cadmium were reported (Segar et al., 1971). This copper concentration is somewhat higher than that obtained in this study, while Segar et al.'s zinc value is much higher. The cadmium concentrations for the two areas are comparable.

Metal concentrations of Mercenaria mercenaria collected in the study area are shown in Table 15. Correlations were run between log metal concentration and log average dry weight for quahogs from Stations 5 and P9. The only significant correlation found was for zinc at Station P9 ($r=-0.763^*$ d.f. 6, $p<0.05$), indicating that smaller quahogs had higher zinc concentrations than larger conspecifics at this station. Romeril (1971) reported positive correlations of zinc and copper concentrations with size in M. mercenaria, and in a

Table 15. Metal concentrations of Mercenaria mercenaria in ppm dry weight.

DRY WT. (g)	CONCENTRATIONS Cu	Zn	Cd	NUMBER OF INDIVIDUALS	AVERAGE DRY WT. (g)
			STATION 5		
1.068	79.6	262.3	0.94	1	1.068
1.561	60.9	297.9	0.96	1	1.561
2.235	67.1	90.4	0.89	1	2.235
0.530	40.1	264.8	2.36	1	0.530
1.454	47.3	158.1	2.06	2	0.727
			STATION P9		
0.726	72.4	186.1	1.61	1	0.726
0.820	21.3	207.2	1.52	4	0.205
0.387	87.2	151.2	1.94	1	0.387
1.394	41.2	156.9	0.90	2	0.697
1.861	70.5	138.4	3.09	1	1.861
2.386	35.6	90.1	0.73	1	2.386
1.439	18.2	119.9	0.87	1	1.439
1.383	16.3	178.9	0.90	1	1.383
			STATION OB		
5.887	27.2	152.9	0.85	1	5.887
10.942	22.8	228.5	1.00	1	10.942
			STATION P17		
0.776	45.1	148.1	1.29	1	0.776
0.463	43.2	155.5	-	2	0.232
1.816	31.7	67.8	0.69	1	1.816

more recent study (1974), found copper concentrations to be negatively correlated with tissue weight in the quahog. Comparison of the mean metal concentrations at the different stations (Table 16) revealed these trends: for copper, Station 5>P17>P9>OB; for zinc, Station 5>OB>P9>P17; for cadmium, Station 5>P9>P17>OB. The only consistent feature was the higher metal concentrations in quahogs from Station 5. The EPA reports that normal metal concentrations in quahogs average approximately 25 ppm Cu, 140 ppm Zn, and 1.5 ppm Cd. Relative to these averages, M. mercenaria from Station 5 had elevated copper concentrations, and elevated zinc concentrations are noticeable in individuals from both Stations 5 and OB. These quahogs are noticeably contaminated with metals, and the taking of shellfish from this area is illegal.

Metal concentrations of some other benthic species are listed in Table 17. The bivalve Anadara transversa had a very high species cadmium concentration, and it exhibited a trend of increased zinc concentrations in individuals from the more polluted sediments of Station 5. The whelk Busycon canaliculatum had a high species zinc concentration. All four crab species had zinc concentrations about 1½ to 2 times their copper concentrations. No noticeable differences in metal concentrations between stations occurred for any crab species. Decapods, particularly crabs, are known to actively regulate their body zinc and copper concentrations (Bryan, 1967a, 1968), which may explain the comparatively constant crab metal concentrations observed. Martin (1974) reported

Table 16. Station mean metal concentrations and 95% confidence limits for Mercenaria mercenaria. Mean quahog metal concentrations are arranged in decreasing order. Corresponding estimated sediment metal concentrations are also listed. All metal concentrations are in ppm dry weight.

STATION	ESTIMATED SEDIMENT METAL CONCENTRATION	N	MEAN TISSUE METAL CONCENTRATION	L_1	L_2
		Cu			
5	2200	5	57.3	40.8	80.3
P17	220	3	39.5	24.5	63.8
P9	160	8	37.8	21.7	65.7
OB	900	2	24.9	22.8*	27.2*
		Zn			
5	750	5	196.8	105.9	365.7
OB	600	2	187.1	152.8*	228.5*
P9	360	8	149.3	119.9	185.9
P17	400	3	115.9	36.4	369.0
		Cd			
5	20.0	5	1.37	0.67	2.36
P9	3.0	8	1.35	0.84	2.00
P17	3.5	2	0.97	0.69*	1.29*
OB	6.5	2	0.92	0.85*	1.00*

* denotes the upper and lower values for metals at stations where only two samples were analyzed, not L_1 and L_2.

Table 17. Metal concentrations of additional species. All metal concentrations are in ppm dry weight.

STATION	DRY WT. (g)	CONCENTRATIONS Cu	Zn	Cd	NUMBER OF INDIVIDUALS
		Anadara transversa (transverse ark)			
5	0.272	115.1	501.8	16.57	2
5	0.287	34.8	513.9	28.70	1
P9	0.793	50.4	214.4	32.16	5
P9	0.328	30.4	199.4	15.22	3
		Busycon canaliculatum (channeled whelk)			
5	2.932	-	1439.0	3.50	1
OB	0.896	429.9	848.7	22.90	1
		Libinia emarginata (spider crab)			
5	25.332	30.4	60.8	0.55	1
5	26.706	54.7	73.4	0.52	1
OB	12.940	42.5	87.3	0.62	1
OB	12.234	54.8	76.8	0.98	1
P9	2.499	60.0	94.1	0.80	1
P9	4.816	52.4	86.3	0.52	2
P17	13.562	-	89.2	0.66	1
P17	17.142	61.3	89.8	0.70	1
		Neopanope texana			
5	2.486	41.2	76.4	0.80	2
5	2.644	60.5	82.5	0.38	3
5	3.769	47.8	69.8	0.53	2
P9	3.054	47.5	65.5	0.49	2
		Cancer irroratus (rock crab)			
5	2.270	72.7	99.6	1.98	1
		Ovalipes ocellatus (lady crab)			
OB	8.906	60.6	116.8	2.47	1

concentrations of 103.0 ± 29.5 ppm Zn and 66.8 ± 37.2 ppm Cu for rock crabs Cancer irroratus in Nova Scotian waters, which are quite similar to those obtained from New Bedford Harbor.

DISCUSSION

There are three possible routes of metal uptake in marine organisms - from food, solution or ingested particulate matter. Which of these routes is the main source of copper, zinc and cadmium for Crepidula fornicata is not known. Inorganic particles and their adsorbed metals are strained from the water along with organic food particulates by the gill filaments of filter feeding bivalves during the feeding process, and a substantial quantity are ingested along with the fine food particles (Preston et al., 1972; Boyden and Romeril, 1974). A similar feeding mechanism has been demonstrated in the gastropod C. fornicata (Orton, 1912). Metal uptake from the ingestion of clay-sized particulate matter is therefore assumed to be a main route of metal assimilation in the filter feeding slipper limpet.

Summerhayes et al. (1977) calculated that approximately sixty per cent of the copper and zinc enters New Bedford Harbor in solution. They also demonstrated that these dissolved metals are rapidly removed from the water column and become incorporated in the bottom sediments. Most of the metal load is therefore in some particulate form and is associated with the clay fraction of the sediment. Subsequent seaward transport of metals out of the inner harbor and into Buzzards Bay is facilitated by eddy diffusion (Summerhayes et al., 1977).

Availability of these metals to the biota depends substantially on the chemical changes the metals undergo as they

are resuspended into the oxidizing sea water and settle back into the reducing bottom sediments. Total sediment metal concentrations do not necessarily reflect the amount of metals available for uptake by marine organisms (Phillips, 1977a). The high uptake of metals by the slipper limpets at Station P17 relative to the estimated sediment metal concentrations there thus may have been due to some unique environmental condition (redox potential, ligands available for metal binding, copresence of other metals, etc.) which increased the biological availability of certain metals. It is also possible that the estimated sediment metal concentrations at Station P17 were inaccurate. Spatial variability of sediment metal concentrations may exist at this location. Overall, there are definite consistent trends in sediment metal concentrations over the study area, as demonstrated in the data of Summerhayes et al. (1977) (see Figures 1 and 2). Yet the possibility of local fluctuations in sediment metal levels remains. Relative to the other stations, slipper limpet metal concentrations at Station P17 were not proportional to the total estimated sediment concentrations of metal. Frazier (1976) also found the relation between sediment and tissue metal concentrations in Crassostrea virginica to be non-linear, yet sediments with the greater metal concentrations usually had oysters with higher metal concentrations.

Other investigators demonstrated that salinity and temperature can have significant effects on the rates of metal uptake by marine organisms (Cunningham and Tripp, 1975b;

Fowler and Benayoun, 1976a; Phillips, 1976a, 1977b; Jackim et al., 1977). In the present study, salinity and temperature differences between the bottom waters of the four stations at any time of the year were insignificant. Salinities usually ranged between 27.5 and 30.8 parts per thousand (ppt) over the year, with an occasional value as low as 24.0 ppt (Summerhayes et al., 1977). Any differential effects of salinity or temperature on metal uptake by C. fornicata of the different stations were therefore considered insignificant.

Several studies have shown no relationship between zinc concentration and body weight in C. virginica (Huggett et al., 1973), tissue copper concentration and whelk size in Busycon canaliculatum (Betzer and Pilson, 1974) or lead concentration and abalone weight (Stewart and Schulz-Blades, 1976). Crepidula fornicata behaves in a similar manner, with no demonstrated correlation between tissue copper, zinc or cadmium concentration and slipper limpet weight.

Storage of cadmium, copper and zinc has been demonstrated in the liver and kidneys of terrestrial mammals (Webb, 1975). These storage products are low molecular weight high cysteine content proteins called metallothioneins; similar metal binding proteins have been found in marine vertebrates (Olafson and Thompson, 1974). Cadmium-thioneins have been shown to have long biological half-lives, while zinc and copper-thioneins have significantly shorter biological half-lives (Webb, 1975). The long half-lives for cadmium-proteins reflect the often observed cumulative property of cadmium.

Webb (1975) suggests metallothioneins may have some physiological function in the control of the metabolism of these metals in addition to the original assumption of their being protective mechanisms against toxic effects of the metals. Limpets Patella spp. were also found to complex cadmium and copper with low molecular weight proteins (Howard and Nickless, 1977). These authors suggested that the ability of the limpets to store cadmium and copper as metallothionein complexes may be the reason for their survival in polluted environments and may help explain the increased metal concentrations accumulated by limpets in fouled waters. Such mechanisms may well be operating for copper, zinc and cadmium in C. fornicata from the New Bedford Harbor area. That this species can proliferate in such heavily polluted waters as New Bedford Harbor makes it an excellent candidate for use as a monitor of metal pollution. Further studies of the different organs and tissues of the slipper limpet may help locate the sites of metal storage. Evidence that the digestive gland/hepatopancreas of crustaceans and molluscs are consistently one of the organs with the highest metal concentrations (Bryan, 1967a; Schulz-Blades, 1974) suggests the probable production and storage of metallothioneins in these areas.

Most previous attempts at utilizing fish (Johnels, Westermark, Berg, Persson and Sjöstrand, 1967; Dix and Martin, 1975) and marine molluscs (Navrot et al., 1974; Alexander and Young, 1976; Phillips, 1976b) in coastal metal

pollution monitoring involved the sampling of animals along many miles of coastline. Sampling stations were considerably further apart in these undertakings than in the present study. Such monitoring programs were used to detect large regions of pollution or to monitor the dispersal of ocean-disposed wastes by currents (Pesch, Reynolds and Rogerson, 1977). The New Bedford Harbor study of C. fornicata was on a smaller scale than these other monitoring studies, and was designed to test whether the slipper limpet had metal monitoring potential. The results indicated that this species has potential for the monitoring of cadmium and zinc pollution. This was seen in the significant positive correlation between the tissue and sediment cadmium and zinc concentrations. Boyden (1975) found a similar correlated decrease in shellfish and sediment metal concentrations with increasing distance from the point of pollution in an English harbor.

Expansion of a monitoring program using C. fornicata over a considerably larger area may further establish the monitoring capacities of this organism for zinc and cadmium and perhaps other metals. Copper pollution monitoring with C. fornicata does not seem advisable, as no significant correlation existed between tissue and sediment concentrations of this metal and the mean copper concentration of slipper limpets from Stations 5 and OB (with the greater sediment metal concentrations) was not significantly different from that of conspecifics from Stations P9 and P17. Copper plays a vital role in the respiratory physiology of molluscs, being

incorporated in the blood pigment hemocyanin (Gardiner, 1972). A significant proportion of the body burden of copper in the slipper limpet may be associated with the blood hemocyanins, as has been demonstrated for Busycon canaliculatum (Betzer and Pilson, 1974). Due to the importance of copper to the survival of C. fornicata, it is doubtless regulated to some extent by the organism. Such regulation may account for the non-significant correlation between slipper limpet and sediment copper concentrations.

New Bedford is a heavily contaminated harbor. The filter feeders C. fornicata and M. mercenaria, occupying low positions in the food chain, accumulate copper, zinc and cadmium and other metals and chemicals (including PCBs) from this environment. Commercially important groundfishes enter this polluted area, consume contaminated molluscs and crustaceans, and then move on, extending the influence of this problem over a much wider geographical area. The New Bedford fishing fleet doubtless harvests fish which have fed in the New Bedford Harbor region. Mercenaria mercenaria itself is an important commercial shellfish, and quahogs are taken regularly from waters at the Dartmouth-New Bedford line and sold to the general public. Constant monitoring of the metal concentrations in these shellfish is advisable for reasons of public health.

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APPENDIX

Calculations.

All correlations performed were to determine the product-moment correlation coefficient (r_{12}),

$$r_{12} = \Sigma y_1 y_2 / \sqrt{\Sigma y_1^2 \Sigma y_2^2}$$ (Sokal and Rohlf, 1969)

where y_1^2 = the sum of squares of variable Y_1 and

y_2^2 = the sum of squares of variable Y_2

Example 1. Correlation of log average dry weight (Y_1) vs log animal zinc concentration (Y_2) for Crepidula fornicata from Station 5.

$$\Sigma Y_1 = .109 + .084 + ... + .011 = .719$$

$$\Sigma Y_1^2 = (.109)^2 + (.084)^2 + ... + (.011)^2 = .0494$$

$$\Sigma Y_2 = 2.081 + 2.237 + ... + 2.071 = 29.248$$

$$\Sigma Y_2^2 = (2.081)^2 + (2.237)^2 + ... + (2.071)^2 = 61.1684$$

$$\Sigma Y_1 Y_2 = .109(2.081) + .084(2.237) + ... + .011(2.071) = 1.5061$$

$$\Sigma y_1^2 = \Sigma Y_1^2 - (\Sigma Y_1)^2/n = .0494 - (.719)^2/14 = .0124$$

$$\Sigma y_2^2 = \Sigma Y_2^2 - (\Sigma Y_2)^2/n = 61.1684 - (29.248)^2/14 = .0651$$

$$\Sigma y_1 y_2 = \Sigma Y_1 Y_2 - ((\Sigma Y_1)(\Sigma Y_2))/n = 1.5061 - ((.719)(29.248))/14 = .004$$

$$r_{12} = \frac{\Sigma y_1 y_2}{\sqrt{\Sigma y_1^2 \Sigma y_2^2}} = \frac{.004}{\sqrt{.0124(.0651)}} = .141 \text{ N.S.}$$

$.05\ (12) = .532$

$.01\ (12) = .661$

Example 2. Correlation of log *Crepidula fornicata* zinc concentrations (Y_1) vs log sediment zinc concentrations (Y_2). Each sample was correlated with the estimated sediment metal concentration of the station from which it came.

$$\Sigma Y_1 = 2.081 + 2.237 + \ldots + 1.952 = 141.541$$

$$\Sigma Y_1^2 = (2.081)^2 + (2.237)^2 + \ldots + (1.952)^2 = 282.600$$

$$\Sigma Y_2 = 2.875 + 2.875 + \ldots + 2.778 = 189.851$$

$$\Sigma Y_2^2 = (2.875)^2 + (2.875)^2 + \ldots + (2.778)^2 = 508.862$$

$$\Sigma Y_1 Y_2 = 2.081(2.875) + 2.237(2.875) + \ldots + 1.952(2.778) = 378.861$$

$$\Sigma y_1^2 = \Sigma Y_1^2 - (\Sigma Y_1)^2/n = 282.600 - (141.541)^2/71 = .433$$

$$\Sigma y_2^2 = \Sigma Y_2^2 - (\Sigma Y_2)^2/n = 508.862 - (189.851)^2/71 = 1.208$$

$$\Sigma y_1 y_2 = \Sigma Y_1 Y_2 - ((\Sigma Y_1)(\Sigma Y_2))/n$$

$$= 378.861 - 141.541(189.851)/71 = .386$$

$$r_{12} = .386/\sqrt{.433(1.208)} = .529^{**}$$

$.05\ (60) = .250$

$.01\ (60) = .325$

Example 3. Correlation of log Crepidula fornicata zinc concentrations (Y_1) vs log sediment zinc concentrations (Y_2) excluding data from Station P17. Each sample was correlated with the estimated sediment metal concentration of the station from which it came.

$$\Sigma Y_1 = 2.081 + 2.237 + \ldots + 1.952 = 79.995$$

$$\Sigma Y_1^2 = (2.081)^2 + (2.237)^2 + \ldots + (1.952)^2 = 160.280$$

$$\Sigma Y_2 = 2.875 + 2.875 + \ldots + 2.778 = 109.189$$

$$\Sigma Y_2^2 = (2.875)^2 + (2.875)^2 + \ldots + (2.778)^2 = 298.979$$

$$\Sigma Y_1 Y_2 = 2.081(2.875) + 2.237(2.875) + \ldots + 1.952(2.778) = 218.718$$

$$\Sigma y_1^2 = 160.280 - (79.995)^2/40 = .300$$

$$\Sigma y_2^2 = 298.979 - (109.189)^2/40 = .923$$

$$\Sigma y_1 y_2 = 218.718 - (79.995)(109.189)/40 = .354$$

$$r_{12} = .354/\sqrt{.300(.923)} = .673^{**}$$

$$.05\ (35) = .325$$

$$.01\ (35) = .418$$

Example 4. Analysis of variance of the mean log zinc concentrations of Crepidula fornicata from different stations (Sokal and Rohlf, 1969).

$$\sum_{a}\sum_{n} Y = 39.217 + 32.938 + 61.546 + 7.840 = 141.541$$

$$\sum_{a}\sum_{n} Y^2 = 81.047 + 63.866 + 122.320 + 15.367 = 282.600$$

$$\sum_{a} \frac{(\sum_{n} Y)^2}{n_i} = (39.217)^2/19 + (32.938)^2/17 + (61.546)^2/31 + (7.840)^2/4 = 282.321$$

$$CT = \frac{(\sum_{a}\sum_{n} Y)^2}{\sum_{a} n_i} = (141.541)^2/71 = 282.167$$

$$SS_{total} = \sum_{a}\sum_{n} Y^2 - CT = 282.600 - 282.167 = .433$$

$$SS_{groups} = \sum_{a} \frac{(\sum_{n} Y)^2}{n_i} - CT = 282.321 - 282.167 = .154$$

$$SS_{within} = SS_{total} - SS_{groups} = .433 - .154 = .279$$

Source of variation	df	SS	MS	F_s
Among groups	3	.154	.051	$\frac{.051}{.004} = 12.37^{***}$
Within groups	67	.279	.004	
Total	70	.433		

$F_{.05(3,60)} = 2.76$ $F_{.01(3,60)} = 4.13$ $F_{.001(3,60)} = 6.17$

A priori tests among the means (Sokal and Rohlf, 1969).

1 SS [for (Stations 5 and OB) vs (Stations P9 and P17)]

$$= \frac{(\Sigma Y_5 + \Sigma Y_{OB})^2}{n_5 + n_{OB}} + \frac{(\Sigma Y_{P9} + \Sigma Y_{P17})^2}{n_{P9} + n_{P17}} - \frac{(\Sigma Y_5 + \Sigma Y_{OB} \cdots + \Sigma Y_{P17})^2}{n_5 + n_{OB} \cdots + n_{P17}}$$

$$= (39.217 + 7.84)^2/(19 + 4) + (32.938 + 61.546)^2/(17 + 31) - (141.541)^2/71 = 96.277 + 185.984 - 282.167$$

$$= .093 = MS$$

$$F_s = MS/MS_{within} = .093/.004 = 23.36^{***}$$

2 SS [for Station 5 vs Station OB]

$$= (39.217)^2/19 + (7.840)^2/4 - (39.217 + 7.84)^2/(23)$$

$$= 80.946 + 15.366 - 96.277 = .036$$

$$F_s = .036/.004 = 8.94^{**}$$

3 SS [for Station P9 vs P17]

$$= (32.938)^2/17 + (61.546)^2/31 - (32.938 + 61.546)^2/48$$

$$= 63.818 + 122.190 - 185.984 = .025$$

$$F_s = .025/.004 = 6.278^{*}$$

Critical values for the above a priori tests:

$F_{.05(1,60)} = 4.00$ $F_{.01(1,60)} = 7.08$ $F_{.001(1,60)} = 12.00$