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Title: ZINC, COPPER AND MANGANESE IN THE RAZOR CLAM, *Siliqua patula*

Signature redacted for privacy.

Abstract approved: Norman H. Cutshall

Zinc, copper and manganese concentrations were measured by atomic absorption spectroscopy in *Siliqua patula* collected during May, 1970 through May, 1971. Individual clams were analysed to determine the degree of variation among individuals. Statistical analysis proved the variation to be highly significant. Monthly composite samples of 18 clams were collected from three sites along the Washington-Oregon coast. The samples were analysed to examine differences in concentration among two general groups of tissue, collection sites and monthly concentrations within a site.

Significant differences were found between the tissue groups, with copper and manganese being consistently higher in portion B (gills, liver, digestive system and reproductive tissue). Zinc was generally found in higher concentration in portion A (foot, neck, mantle, adductor muscle and reproductive tissue).

A statistically significant difference was also noted between the three sites, however no pattern in the concentration was apparent. These results may indicate that the variation between sites reflects only the high degree of variability among individuals.

Statistically significant differences were also noted between the months examined within each site. There appeared to be a general trend of increasing concentration in Spring and Summer for all three elements. Prior investigations indicate that the razor clam undergoes most of its growth activity in early Spring and most of its reproductive activity in late Spring and early Summer. It is possible that the increased concentration in Spring and Summer is responding to increased requirements due to changes in metabolic rate brought about by increased growth and the reproductive activity. However it is equally important to recognize that the monthly variation could be reflecting the variation among individuals.

Zinc, Copper and Manganese in the Razor Clam, *Siliqua patula*

by

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INTRODUCTION

The Northern Razor Clam, *Siliqua patula* Dixon 1788, inhabits broad, sandy beaches from central California to Alaska (Fitch, 1953). Its depth distribution ranges from the low tide mark seaward to a depth of 30 fathoms (McMillin, 1924). *Siliqua patula* is a filter feeding organism utilizing planktonic organisms primarily *Chaetoceros armatus*, an inshore diatom, as its major food source for at least six months (October to April) of the year (Tegelberg and McGoon (1969). This type of feeding behavior would provide the most probable pathway for the entrance of particulate inorganic material. Prior to this paper no direct evidence of the extent of accumulation of trace elements by the razor clam was available. However studies of other members of the order, Teleodesmacea, indicate an accumulation of zinc, copper and manganese (Vinogradov, 1953). It is also well known that many marine species concentrate certain transition elements such as manganese, zinc, copper, iron, cobalt and nickel (Lowman, 1960). The purpose of this thesis is to establish average concentration values for certain of these essential trace elements namely zinc, copper and manganese in *Siliqua patula* with regard to geographical location, season and general location within the soft body tissue of the organism.

Biological factors such as feeding habits, metabolic rate, growth rate, life span, nutritional status of the element, reproductive rate and surface area to biomass can affect both the uptake rate and extent of accumulation of trace elements. In addition such physical factors as temperature, physical state (ionic or particulate) of the element, chemical competition, distribution and availability can also affect the uptake rate and accumulation of trace elements from the marine environment.

Since zinc, copper and manganese are essential trace elements especially with regard to many enzymatic reactions it is possible that the uptake of these trace elements is associated with metabolic activity. Tegelberg (1964) demonstrated that the growth rate of <u>Siliqua patula</u> was lowest in the winter with a rapid increase occurring in late February and early March. Such activity indicates a change in the metabolic rate. Later in the spring, during the last of April and May, razor clams undergo most of their spawning activity which could also affect the metabolism of the organism (Tegelberg and McGoon, 1969; Anonymous, 1963; and Washburn, 1900). A change in the rate or extent of accumulation of essential trace elements, coincident with these two changes in metabolic activity might be expected. Taylor (1959) indicates that the growth parameters of <u>Siliqua patula</u> are quantitatively associated with the mean annual air temperatures ranging from California to Alaska. There appears to be an increase in size associated with a corresponding decrease in air temperature. Thus this change in the growth rate with geographical location could again affect the extent to which razor clams concentrate zinc, copper and manganese.

Zinc, Copper and Manganese in the Marine Environment

Bowen (1966) and Goldberg (1963) list an average value of 0.01 micrograms of zinc per gram of seawater. However Spencer et al. (1970) list an average value of 0.003 micrograms of zinc per gram of seawater in the northeast Pacific. The concentration of zinc in various marine species ranges from 6-1500 micrograms per gram of dry weight (Bowen, 1966 and Goldberg, 1963). For molluscans an average zinc concentration of 200 micrograms per gram of dry soft body tissue has been established (Bowen, 1966). The chemical forms of many trace elements found in seawater have not been conclusively established as yet. Rice (1961) suggested that zinc is mostly in a combined form with either sulfur or oxygen. Later evidence indicates that Zn^{2+} and $Zn(OH)_2$ are the principal chemical forms in seawater (Zirino and Healy, 1970). It has been shown that the upper few centimeters of sediment serve as a reservoir for zinc in estuaries (Renfro, 1971). The function of zinc in relation to metabolism is catalytic in nature (Allaway, 1965). It is essential for many of the reactions involving metalloprotein and metalloflavin enzymes (Bowen, 1966).

A classical view of the chemical form of manganese in seawater is that a large portion of the soluble Mn^{2+} found in freshwater becomes oxidized in seawater and deposited in the insoluble form of MnO_2 (Bryan and Ward, 1965). Thus the highest concentration of manganese encountered by marine organisms might be in the areas near the mouth of a river. Rona, et al. (1962) found that manganese in seawater was non-dialyzable in a 0.48 Å dialysis bag indicating that the manganese could be associated with high molecular weight compounds. As with zinc, there are insufficient data on the principal chemical forms of manganese in seawater. However, the concentration of manganese in seawater has been estimated to be 0.002 micrograms per gram of seawater (Bowen, 1966 and Goldberg, 1963). The concentration of manganese in molluscan dry tissue has been estimated at an average of ten micrograms per gram of dry tissue (Bowen, 1966). As with zinc, manganese is an essential trace element serving primarily a catalytic function in regard to metabolism (Allaway, 1965). Its most important role is that of an activator in enzyme systems, principally with isocitrate dehydrogenase in the Kreb's Cycle and many of the reactions involving metalloflavin enzymes (Bowen, 1966).

The concentration of copper in seawater has been estimated at

0.003 micrograms per gram (Bowen, 1966 and Goldberg, 1963). However Spencer et al. (1970) list an average value of 0.002 micrograms per gram of seawater in the northeast Pacific. Again there are relatively few data on the chemical forms. Goldberg (1963) suggests that the principal forms are Cu^{2+} and $CuSO_4$ while Sillén (1961) proposes that $CuOH^+$ is the major form along with Cu^{2+} and the contribution by the $CuSO_4$ form is negligible. Slowey, Jeffrey and Hood (1967) suggest that there is a copper complex associated with the amino lipid, porphyrin or phospholipid portion of lipids. Bowen (1966) lists a value of 20 micrograms of copper per gram of dry tissue for molluscans. Copper is an essential trace element that functions primarily as an activator in reactions involving metalloporphyrin and metalloprotein enzymes in particular cytochrome oxidase (Bowen, 1966).

Previous investigations have shown that marine organisms concentrate trace elements far in excess of the required level. Pequenat, Fowler and Small (1969) calculated a value of 2.7 parts per million for the maximum zinc requirement for live marine organisms. They further indicated that the concentration of zinc in both marine plants and animals varies considerably but in all cases is much higher than the calculated requirement for zinc. Allaway (1965) and Bowen (1966) indicate that zinc, copper and manganese exhibit some degree of toxicity at high concentrations but the dietary levels required to demonstrate toxic effects are extremely high. However, Nielson and Wium-Andersen (1970) found inhibition of growth and photosynthesis in unicellular algae by copper at one to five micrograms of copper per liter.

Table 1. Concentration factors for zinc, copper and manganese in shellfish (Weaver, 1967).

	Concentration factor
Zinc	1, 000-10, 000
Copper	5, 000-50, 000
Manganese	5, 000-50, 000

METHODS AND MATERIALS

Razor clams were collected each month during extreme low tides from May, 1970 through May, 1971. Three sites along the Washington-Oregon coast were sampled (Figure 1). The northernmost site was Roosevelt Beach approximately 22 miles north of Aberdeen, Washington. The second site was approximately 13 miles north of Long Beach, Washington at Oysterville and the southernmost site was five miles south of Astoria, Oregon at Sunset Beach. Eighteen clams per collection site per month were taken in accordance with the maximum sport limit in Washington. However this number was not always obtained due to restrictions set by the Washington State Fisheries Department during the summer months and occasional inclement weather and poor surf conditions during some of the winter months.

A preliminary study was conducted to determine the extent of individual variation in metal content using 20 clams collected at Sunset Beach in May, 1970. Thereafter tissues from all 18 clams from a given site were composited after dissection.

Upon collection the clams were placed on ice and transported to the laboratory to be prepared for analysis. The shells were removed and discarded. Next, the soft body tissue was thoroughly washed in distilled deionized water to remove sand particles and

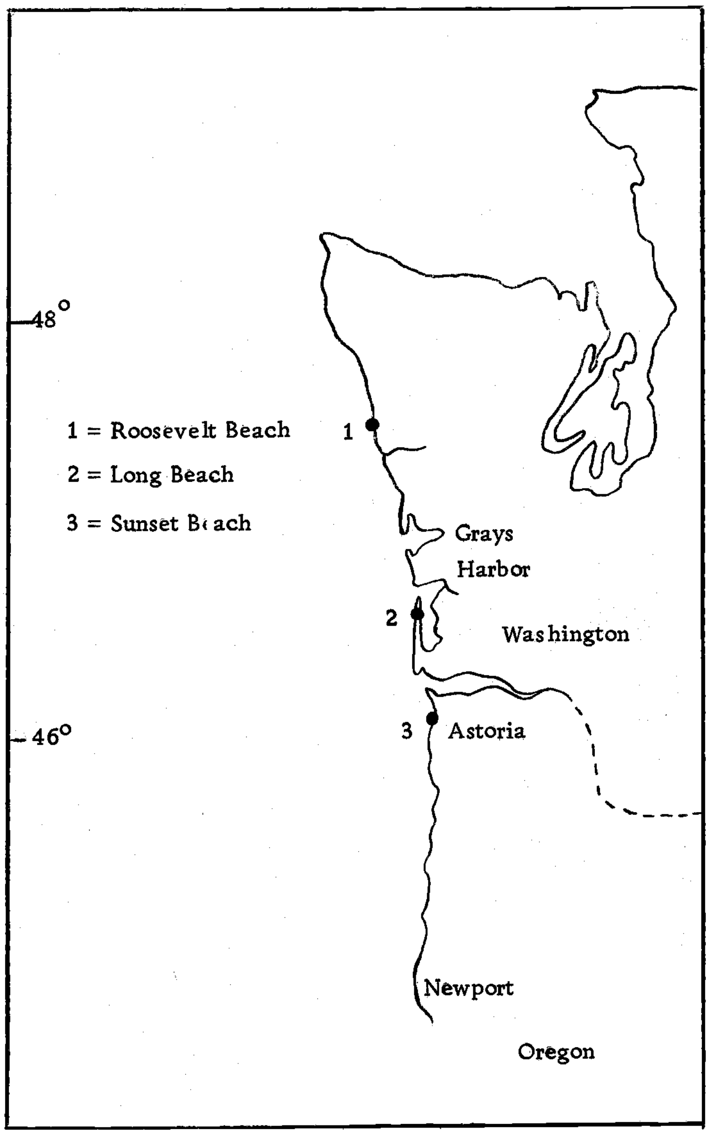

Figure 1. Map of collection sites

then dissected into two portions, A and B. Portion A included the foot, neck, mantle and adductor muscles while portion B included the gills, liver and digestive system. Because the reproductive organs were indistinguishable, except during the spawning period, this tissue may be present in both portions. After division into the two portions, samples were then placed in a vented drying oven at 60 C and allowed to dry to constant weight. After reaching a constant weight each portion was weighed on an analytical balance for a dry weight measurement. The samples were then placed in a muffle furnace and the temperature was gradually increased to 500 C over a period of two hours to avoid a loss of material due to sudden combustion. The samples remained in the muffle furnace for 72 hours by which time they had been reduced to a gray to white ash. Each ash sample was weighed again to establish an ash to dry weight ratio. In the next step 0.1 grams and 0.02 grams of ash were placed in 50 milliliter volumetric flasks. The larger subsample was analysed for copper and manganese while the smaller was analysed for zinc. Two milliliters of 70% HNO_3 were added to each flask and the solution was evaporated to dryness on a hot plate. The flasks were then cooled to room temperature, 20 milliliters of 0.36 N HCl were added and the flasks were returned to the hot plate to boil for a short time. After allowing the flasks to cool, 0.36 N HCl was added to bring the volume to 50 milliliters. Elwell and Gidley (1962) indicate that there is very little chemical interference during analysis from the presence of a dilute acid.

As a means of comparison, working standards were prepared from stock solutions. The zinc standards ranged from 0.5 parts per million to three parts per million while both of the copper and manganese standards ranged from one part per million to 20. Standards were calibrated against N.B.S. standards.

The samples were next analysed for their concentrations of zinc, copper and manganese using a Perkin-Elmer Model 303 atomic absorption spectrophotometer. A single element hollow cathode tube was used in the zinc analysis, while a multielement cathode tube containing copper, iron, manganese, cobalt and nickel was used in the analysis for copper and manganese.

A calibration curve was established using standard solutions prior to every analysis. The curve was not totally linear, usually a slight curvature toward the concentration axis existed resulting from variations in absorption, in different parts of the flame and resonance broadening (Elwell and Gidley, 1962). A total of 12 readings expressed as concentration in parts per million were taken for each element in every sample: six from the sample, four background and two standard readings. These values were then converted to micrograms of element per gram of ash by the following formula:

$$\text{Micrograms/gr. ash wt.} = \begin{matrix}\text{atomic}\\ \text{absorption}\\ \text{reading}\end{matrix} \times \frac{\text{wt. of solution (organism)}}{\text{wt. of dissolved ash (organism)}}$$

Atomic absorption spectroscopy was chosen as the method of analysis primarily because of the ease with which the samples could be handled and the potential accuracy that can be obtained (Ramirez-Muñoz, 1968 and Robinson, 1966).

There is little interelement interference among zinc, copper and manganese when a pre-mixed air-acetylene flame burner is used as in this analysis (Slavin, 1966-67 and 1968). Copper can cause interference with zinc absorption, however this can be eliminated by the use of a single element zinc cathod tube (Slavin, 1968).

Problems can arise in trace element analysis beginning with the initial preparation of the sample if precautionary measures are not taken, Stainless tools rinsed in distilled deionized water were used for the tissue dissection. During drying contamination can occur from air-borne particles for calcium, magnesium, copper, zinc, and aluminum (Thiers, 1957). To minimize this the drying oven was placed in an enclosed hood. Elements that are volatile can be lost if an extremely high temperature is used in the dry ashing process. For this reason the temperature of the muffle furnace was kept at a maximum of 500 C. Problems can also arise in the dissolution of the sample from air contamination, contaminated reagents, loss from evaporation and extraction of contaminating elements from containers. To avoid these problems all containers were rinsed in 70% HNO_3 prior to any preparation. Reagent grade solvents were used for dissolution and the dissolved samples were stored in closed flasks until analysis.

In addition to errors that can occur in the preparation stages there are several sources of error associated with any atomic absorption unit that vary in name and importance with the author. Crow, Hime and Connally (1967) identify four sources: the flame, photocell, matrix and spectral interferences characteristic of atomic absorption and the noise level of the electronic circuitry. They indicate that under optimum conditions errors from these sources can be held to within 1 or 2%.

Skogerboe (1969) identifies two types of error associated with atomic absorption spectroscopy that he labels as (1) random and (2) systematic. The random error will be present in the measurements while the systematic error refers to a unidirectional error or an analysis that is most frequently biased in the same direction. Random sampling will eliminate systematic error at the sampling stage. Loss of the analyte or contamination are two systematic errors that can occur during pretreatment, however these can be controlled by standardization of the techniques involved. Chemical and physical interferences are the prime causes of systematic error at the measurement stage. These may also be compensated for by standardization of the methods involved. Chemical interferences can also be controlled by using a premixed gas or laminar flow burner (Rains, 1969).

Kahn (1968) designated three types of interferences (1) chemical which results from incomplete dissociation of the compounds of the elements being determined, (2) ionization which results when a substantial proportion of the atoms in the sample become ionized causing them to absorb at a different radiation line and (3) matrix interferences are changes in the analytical result caused by the viscosity or nature of the sample solution.

Weberling and Cosgrove (1965) identify chemical interference from substances within the sample, that will form compounds with the element in question that are not readily dissociated, as the most serious interference or source of error.

Even though it is difficult to assign a quantitative error value to the different areas of this study, it is the author's opinion that the preparation stages, involving drying, ashing and dissolution, were the major source of error.

RESULTS

Preliminary Study

Mean values for zinc, copper and manganese in micrograms per gram ash and the associated F test values, for the 20 individual clams examined during the preliminary study, obtained from a one/two Factor Analysis of Variance program are shown in Table 2. The F test statistic used to examine the significance of the variation between individuals was calculated from the following formula:

$$F = \frac{\text{factor A mean square}}{\text{error mean square}}$$

where both mean square values are taken from an Analysis of Variance table. It is evident from a comparison of the calculated F and the tabular F value of 1.88 at the 0.05 level with 19 and 40 degrees of freedom that there was significant variation in the concentration of zinc, copper and manganese among the individual clams examined.

The original data in micrograms per gram ash were converted to micrograms of trace metal per gram of dry weight by multiplying the original data for each clam by its ash to dry weight ratio. The purpose of this conversion was to determine if the high variation observed among individuals was due to the varying amounts of sand associated with the ash. The means and F values obtained from the

Table 2. Comparison of mean values of zinc, copper and manganese in portions A and B in 20 individual razor clams (µg/g. ash).

	Zinc		Copper		Manganese	
Clams	A	B	A	B	A	B
1	550	650	123.3	165.8	108.3	215.0
2	508	566	123.3	168.3	100.0	200.8
3	633	600	92.5	157.5	87.5	180.0
4	658	641	123.3	162.5	154.2	182.5
5	633	491	105.8	154.2	94.2	197.5
6	533	675	101.6	160.0	140.2	263.3
7	716	816	113.3	192.5	152.5	265.8
8	550	858	112.5	174.0	154.2	171.6
9	683	433	95.8	161.6	183.3	225.0
10	558	475	98.3	194.2	149.2	133.3
11	600	691	88.3	162.5	75.0	240.0
12	741	550	101.6	140.0	139.2	158.3
13	616	550	82.5	136.6	85.8	158.3
14	650	650	80.8	139.2	65.8	147.5
15	633	541	101.6	145.8	77.5	190.8
16	750	458	120.5	182.5	150.0	183.3
17	775	666	102.5	140.0	119.2	226.6
18	708	616	107.5	187.4	123.3	200.0
19	641	716	117.5	197.5	60.0	215.8
20	658	541	130.0	195.8	154.2	245.8
Calc. F	9.95**	24.48**	11.71**	11.95**	52.99**	32.07**
Tabular F @ 0.05 19,40 D.F.	1.88	1.88	1.88	1.88	1.88	1.88

one/two Factor Analysis of Variance program are shown in Table 3. Again from a comparison of the calculated and tabular F values at the 0.05 significance level with 19 and 40 degrees of freedom a significant difference in the concentration of zinc, copper and manganese between individuals is evident. These results indicate that the high variation observed among individual clams was not reflecting the varying amounts of sand associated with the ashed sample.

Figures 2 through 7 indicate graphically the degree of variation in the concentration of the three elements for the 20 individuals examined.

Main Study

The overall mean values for each of the three elements in portions A and B at each site were compared by use of the Student T test for paired data to determine if a significant difference in the concentrations of the three elements existed between the two groups of tissue. The T statistic was calculated from the following formula:

$$T = \frac{(\overline{D}-\mu_D)}{S_{\overline{D}}}$$

where $\overline{D}$ = mean difference between the two groups of tissue

$\mu_D = 0$

Table 3. Comparison of mean values of zinc, copper and manganese in portions A and B in 20 individual razor clams (µg/g. dry wt.).

	Zinc		Copper		Manganese	
Clams	A	B	A	B	A	B
1	72.82	86.06	16.32	21.95	14.34	28.46
2	67.3	75.03	16.32	22.28	13.24	26.59
3	83.85	79.44	12.24	20.84	11.58	23.83
4	87.16	84.95	16.32	21.51	20.41	24.16
5	83.85	65.09	16.28	20.40	12.46	26.15
6	70.61	89.37	13.45	21.18	18.64	34.86
7	94.88	108.1	15.0	25.48	20.18	35.19
8	72.82	113.6	14.89	23.03	20.40	22.73
9	90.47	57.37	12.75	21.40	24.27	29.79
10	73.92	62.89	13.01	25.7	19.74	17.65
11	79.44	91.57	11.69	21.51	9.92	31.77
12	98.19	72.82	13.45	18.53	18 42	20.96
13	81.64	72.82	10.92	18.09	11.36	20.96
14	86.06	86.06	10.70	18.42	8.71	19.52
15	83.85	71.72	13.46	19.30	10.26	25.26
16	99.3	60.68	15.95	24.16	19.86	24.26
17	102.59	88.26	13.56	18.53	15.77	30.01
18	93.78	81.64	14.23	24.82	16.32	26.48
19	84.95	94.88	15.55	26.14	7.94	28.57
20	87.16	71.72	17.20	25.92	20.41	32.54
Calc. F.	9.95**	24.49**	12.24**	11.95**	53.01**	32.04**
Tabular F @ .05 19, 40 D.F.	1.88	1.88	1.88	1.88	1.88	1.88

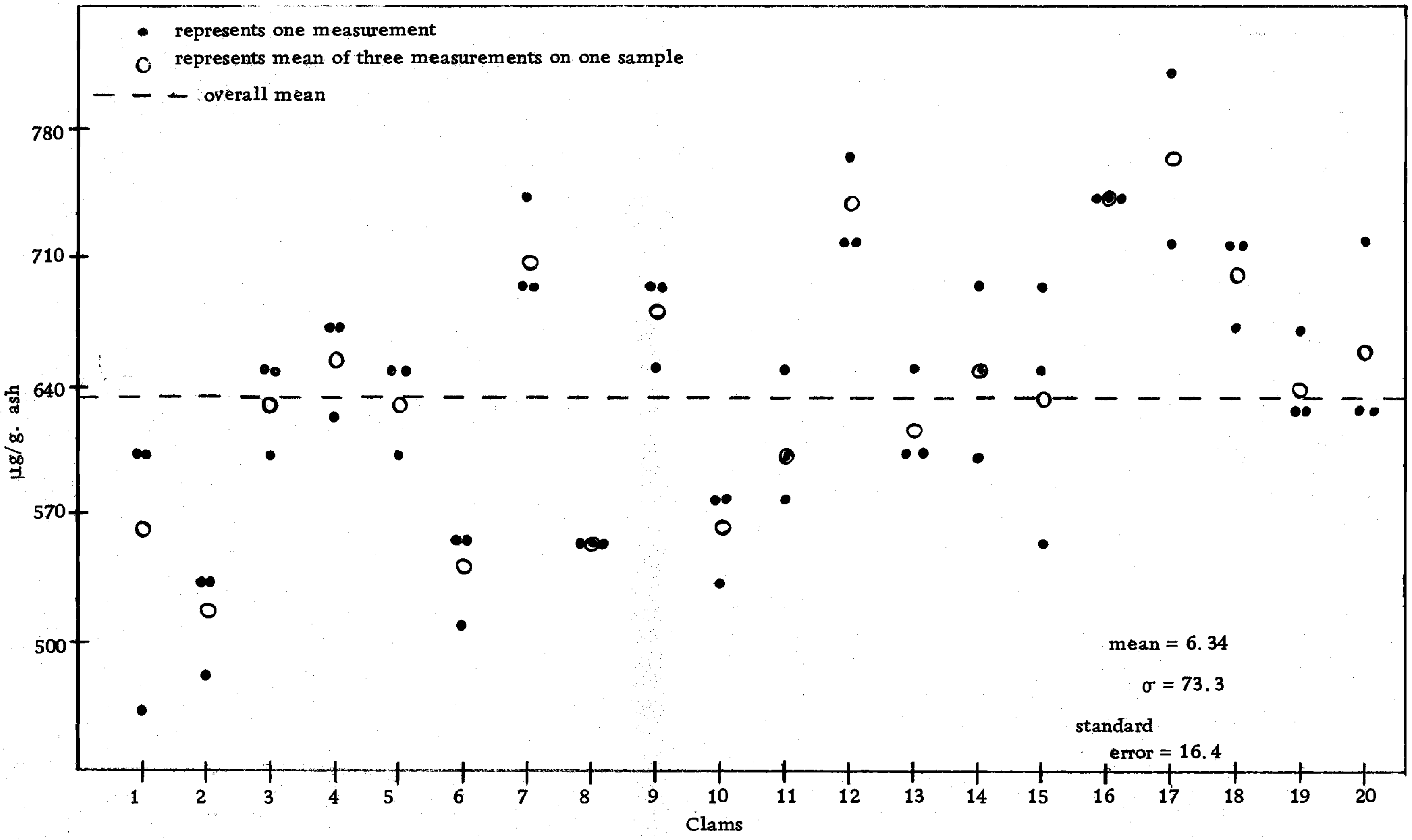

Figure 2. Zinc contents in portion A (foot, neck, mantle, adducter muscles and reproductive tissue) of individual specimens of S. patula.

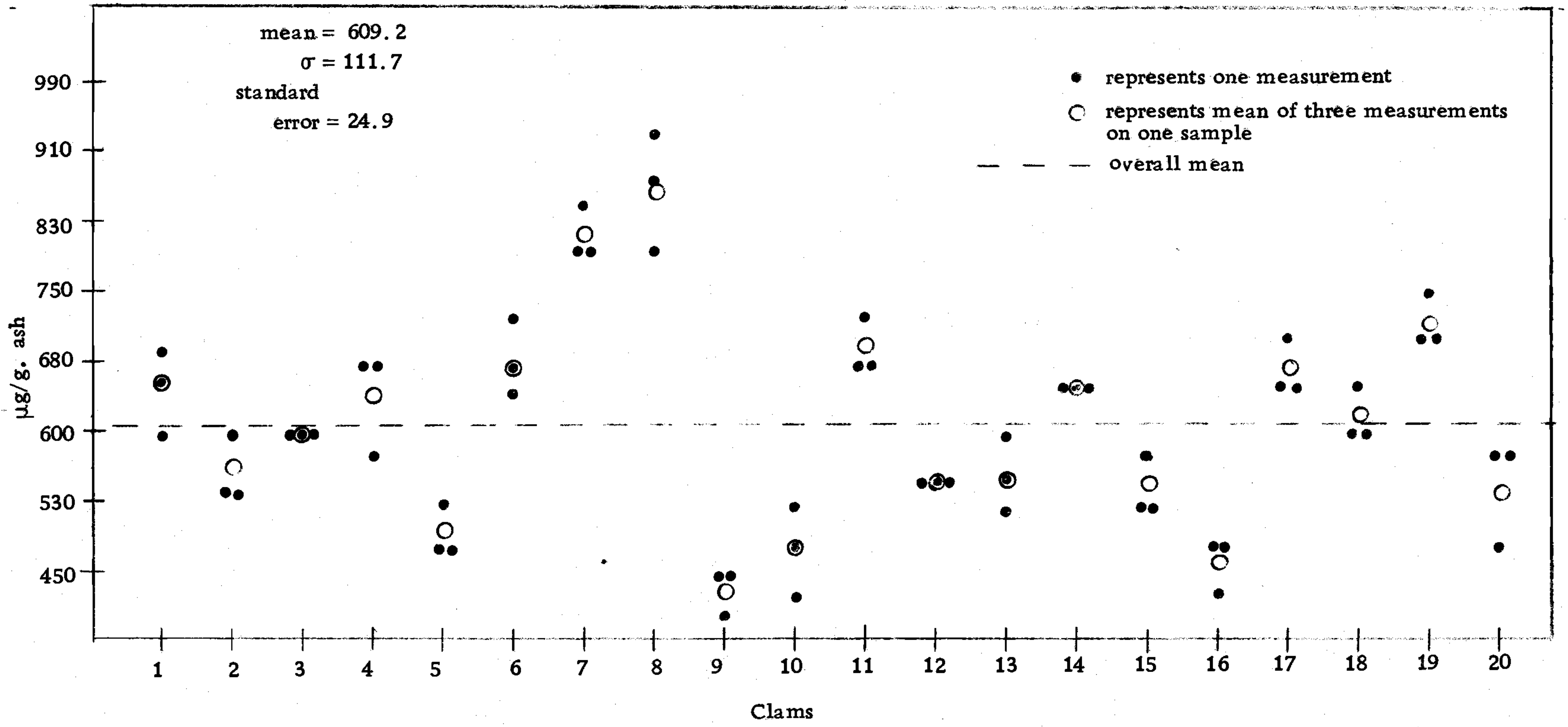

Figure 3. Zinc contents in portion B (gills, liver, digestive system and reproductive tissue) of individual specimens of S. patula.

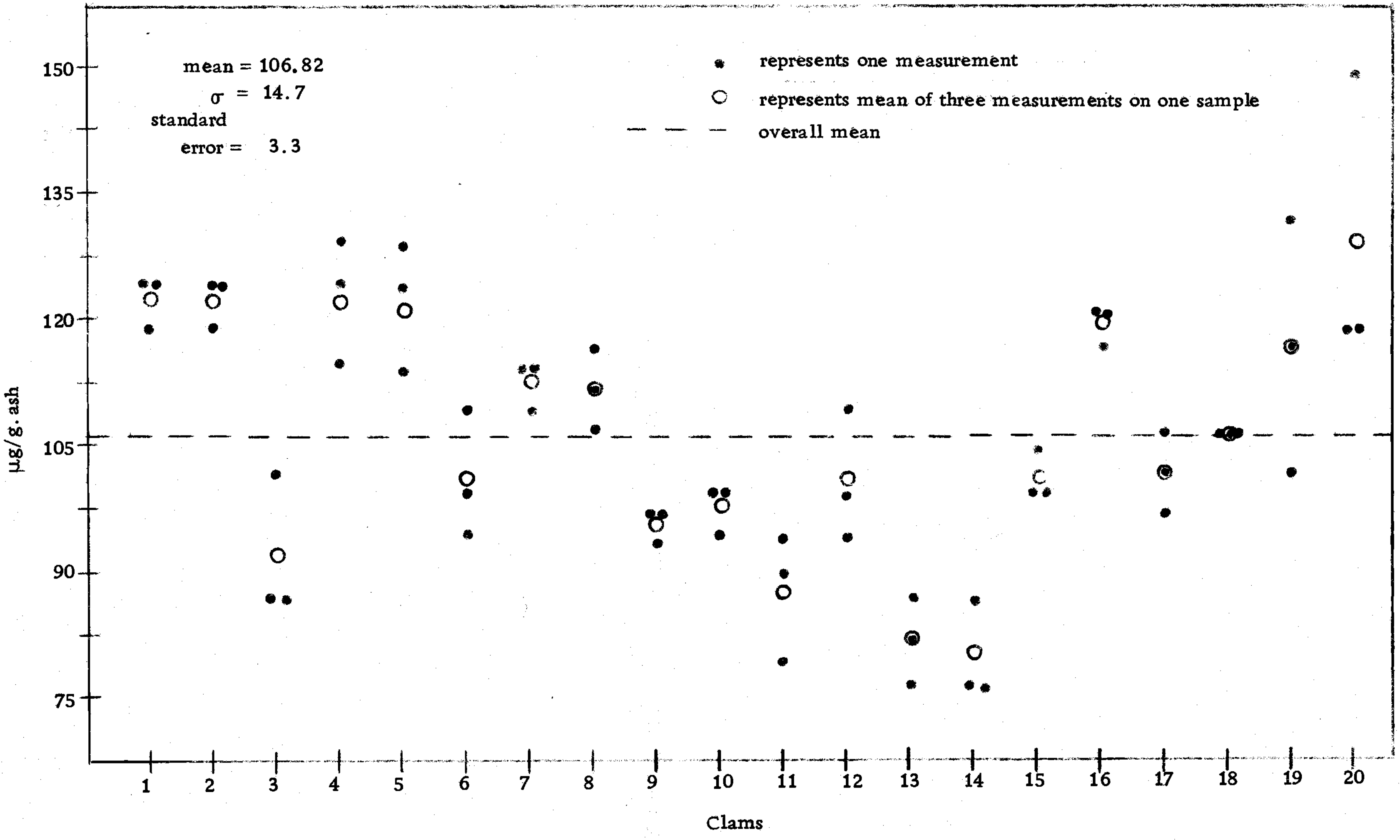

Figure 4. Copper contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) of individual specimes of S. patula.

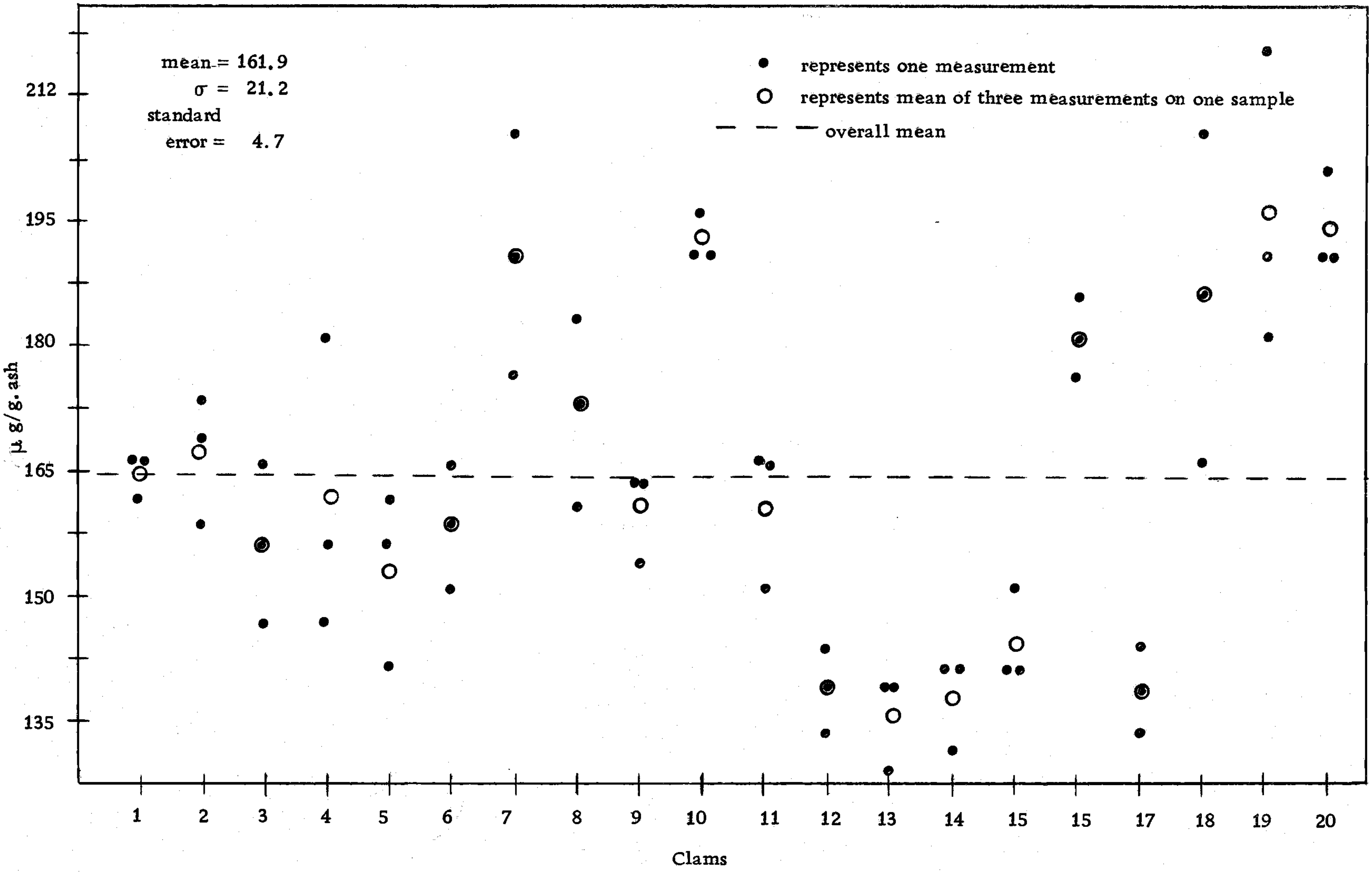

Figure 5. Copper contents in portion B (gills, liver, digestive system and reproductive tissue) of individual specimens of *S. patula.*

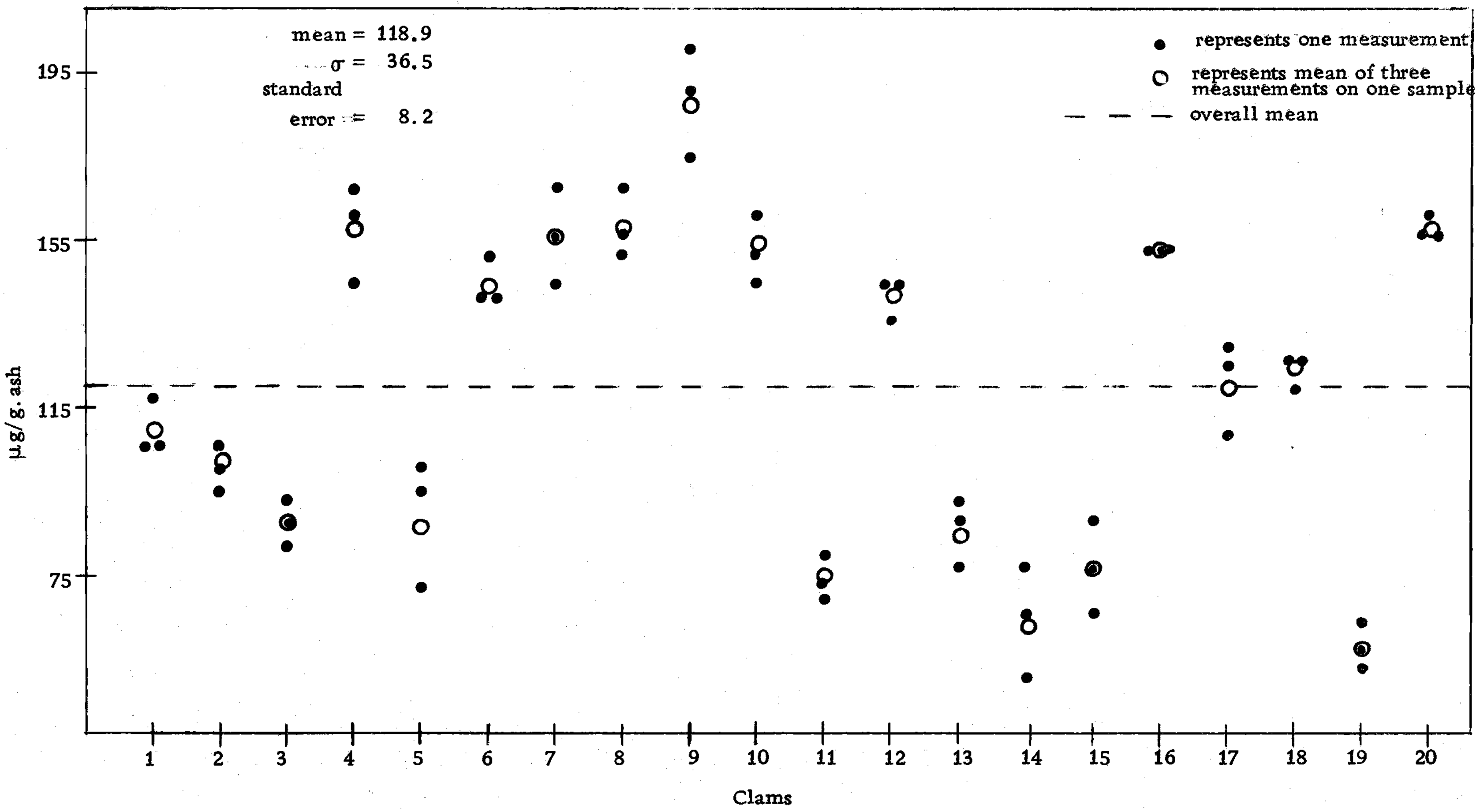

Figure 6. Manganese contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) of individual specimens of S. patula.

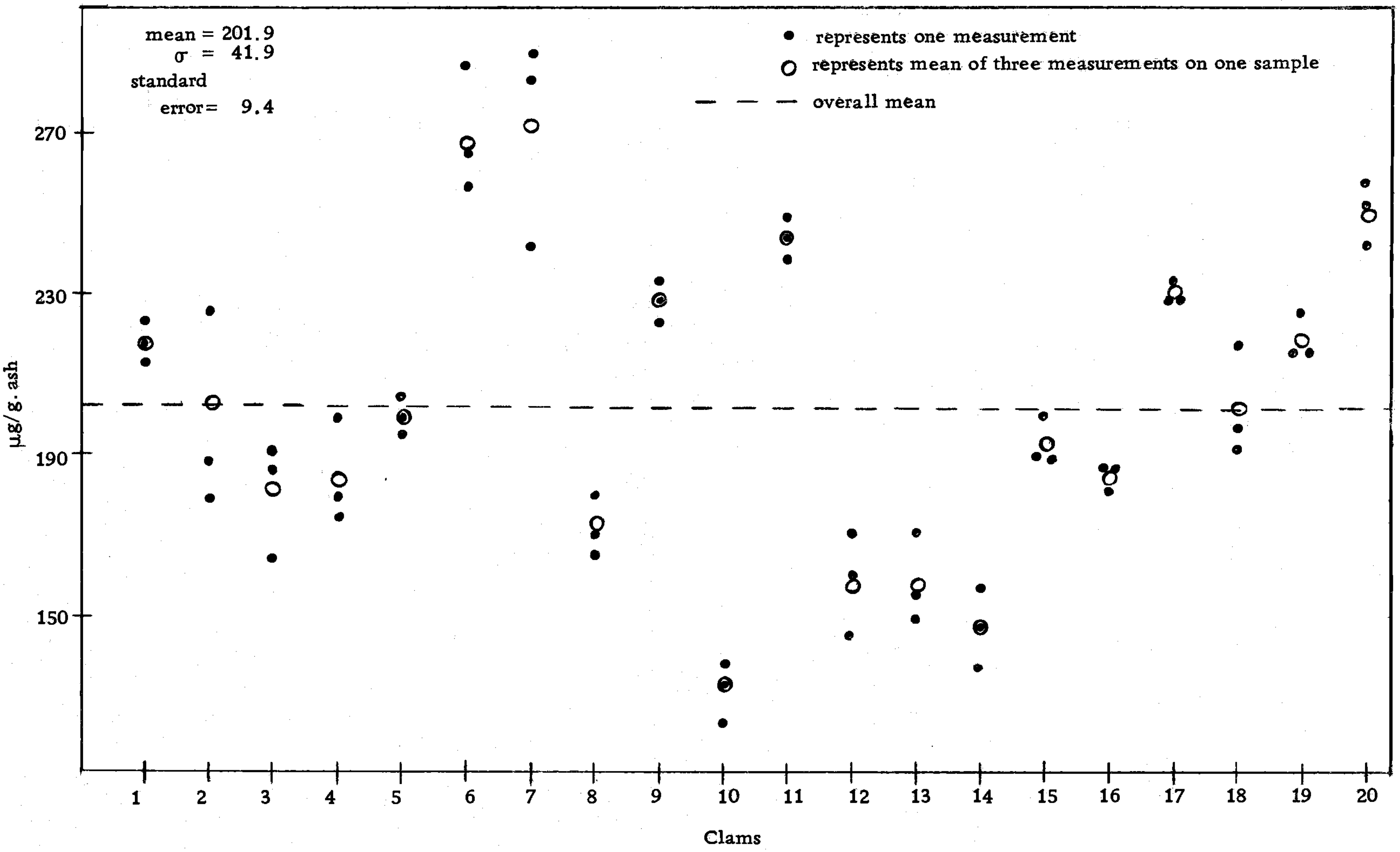

Figure 7. Manganese contents in portion B (gills, liver, digestive system and reproductive tissue) of individual specimens of S. patula.

$$S_{\overline{D}} = S_D / \sqrt{N}$$

$$S_D = \sqrt{\frac{(D_i - \overline{D})^2}{N-1}}$$

N = number of observations (Snedecor and Cochran, 1969).

From a comparison of the calculated T and a tabular T value at the 0.05 significance level with N-1 degrees of freedom it was found that there was a significant difference in the concentration between portions A and B at each site for all three elements. It is also evident from an examination of Tables 4, 5 and 6 that the monthly mean values of copper and manganese are consistently higher in portion B. Further examination of these tables indicate a higher zinc concentration in portion A for all months except May at Sunset Beach and August at Roosevelt Beach.

The concentration of zinc, copper and manganese in portions A and B was compared among the three sites, by the use of F test values generated by the General Linear Hypothesis program, to determine if a significant difference existed between the three sites. The calculated F test values were greater than the tabular F value at the 0.05 level indicating that there was a statistically significant difference in the concentration of zinc and copper in portion A for the three sites examined. The calculated F value for manganese in portion A was found to be less than the tabular F at the 0.05 level

Table 4. Mean values of zinc, copper and manganese in portions A and B from monthly samples at Sunset Beach (µg/g. ash).

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
May 1970	652.5	865.0	54.0	128.5	96.5	177.0
June	975.0	740.0	53.0	113.0	82.5	177.5
July	1485.0	882.5	60.0	145.0	119.5	229.0
August	1020.0	912.5	35.0	160.0	49.0	464.0
September	1027.0	705.0	43.0	148.0	47.5	279.0
October	832.5	712.5	22.5	101.5	27.0	143.0
November	1150.0	617.5	24.0	54.5	36.5	170.5
December	905.0	557.5	23.0	86.0	65.0	276.5
January 1971	*NC	NC	NC	NC	NC	NC
February	NC	NC	NC	NC	NC	NC
March	NC	NC	NC	NC	NC	NC
April	1245.0	1010.0	32.5	124.0	87.0	258.0
Overall mean	1032.44	778.05	38.55	117.83	67.83	241.61
Std. Deviation	241.83	148.26	14.52	33.34	30.61	97.19

*None Collected

Monthly values represent a mean of six observations from a composite tissue sample of 18 razor clams.

Table 5. Mean values of zinc, copper and manganese in portions A and B from monthly samples at Long Beach, Washington (μg/g. ash).

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
June 1970	1030.0	865.0	48.0	163.5	90.5	275.5
July	1367.5	650.0	67.5	90.0	111.5	319.0
August	1157.5	675.0	45.0	135.0	32.0	332.0
September	1085.0	740.0	47.0	171.0	52.5	305.5
October	1025.0	757.5	31.0	108.5	30.0	310.0
November	1093.7	685.0	26.5	136.5	46.5	340.0
December	1050.0	737.5	29.0	133.0	45.0	296.5
January 1971	1337.5	687.5	71.0	137.0	50.0	417.5
February	*NC	NC	NC	NC	NC	NC
March	1160.0	857.5	52.5	113.0	139.0	346.5
April	1165.0	1040.0	37.0	122.0	114.0	231.5
May	1033.3	924.2	51.6	121.6	118.2	434.2
Overall mean	1136.77	767.02	46.01	130.10	75.38	328.02
Standard deviation	119.15	138.08	14.63	23.27	39.71	58.10

*None Collected

Monthly values represent a mean of six observations from a composite tissue sample of 18 razor clams.

Table 6. Mean values of zinc, copper and manganese in portions A and B from monthly samples at Roosevelt Beach, Washington (μg/g. ash).

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
June 1970	845.0	640.0	36.5	114.0	76.5	197.5
July	1380.0	925.0	58.5	189.5	98.0	241.5
August	1027.5	1052.5	25.0	150.0	70.5	251.0
September	1040.0	705.0	55.0	149.0	50.0	232.0
October	962.5	697.5	18.0	88.5	35.5	191.5
November	1075.0	947.0	37.0	163.5	50.5	202.5
December	*NC	NC	NC	NC	NC	NC
January 1941	1250.0	747.5	51.0	184.0	55.0	150.0
February	1025.0	907.5	42.0	130.5	43.0	132.0
March	1250.0	1075.0	44.0	123.0	121.0	336.5
April	1360.0	1072.5	45.0	131.0	105.5	245.0
May	915.0	895.8	73.2	141.6	134.0	355.8
Overall mean	1102.73	878.66	44.11	142.24	76.32	230.46
Standard deviation	180.05	158.56	15.43	27.76	33.57	68.66

*None Collected

Monthly values represent a mean of six observations from a composite tissue sample of 18 razor clams.

indicating that the difference in the manganese concentration existing between the three sites was not statistically significant. When the calculated F values for all three elements in portion B were compared with the tabular F values at the 0.05 level they were found to be greater. These results indicate that the concentration of zinc, copper and manganese in portion B differed significantly between the three collection sites. While a statistically significant difference was generally noted between the three sites there did not appear to be any trend associated with this difference.

To examine any variation between the monthly samples within one site F test values generated by the General Linear Hypothesis computer program were used. It was found from a comparison of the calculated F values, associated with the monthly variation, with the tabular F values at both the 0.05 and 0.01 significance levels that zinc, copper and manganese concentration varies significantly from month to month within each site.

An examination of Figures 8 through 13 indicates a general trend of increasing zinc concentration in both tissue groups during Spring and early Summer. However some random fluctuations appear to exist throughout the rest of the year making it difficult to locate a definite trend with any degree of certainty. From an examination of Figures 14 through 19 this same general condition appears to exist for copper. In regard to the manganese concentration in

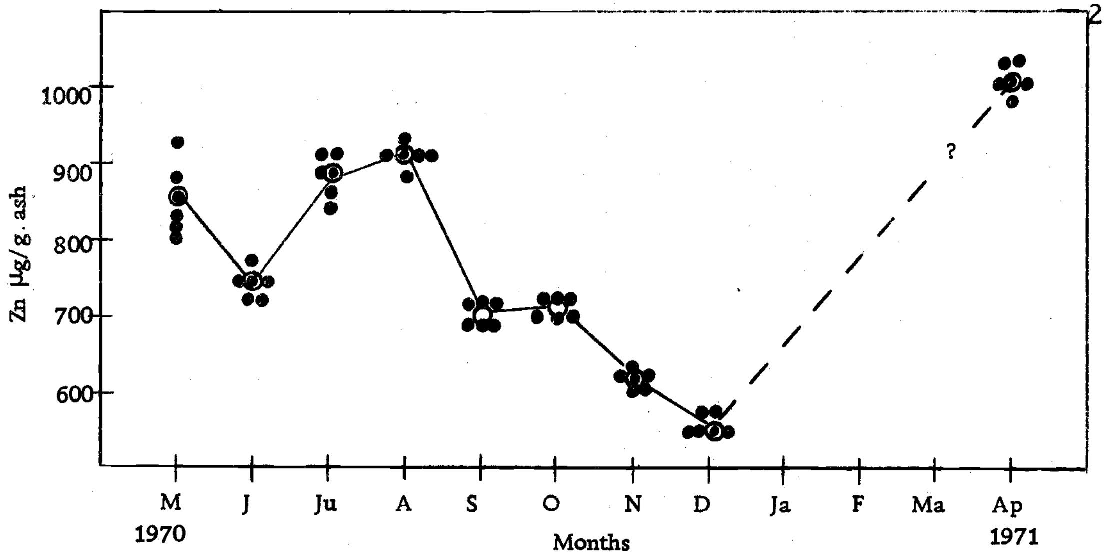

Figure 8. Zinc contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March *

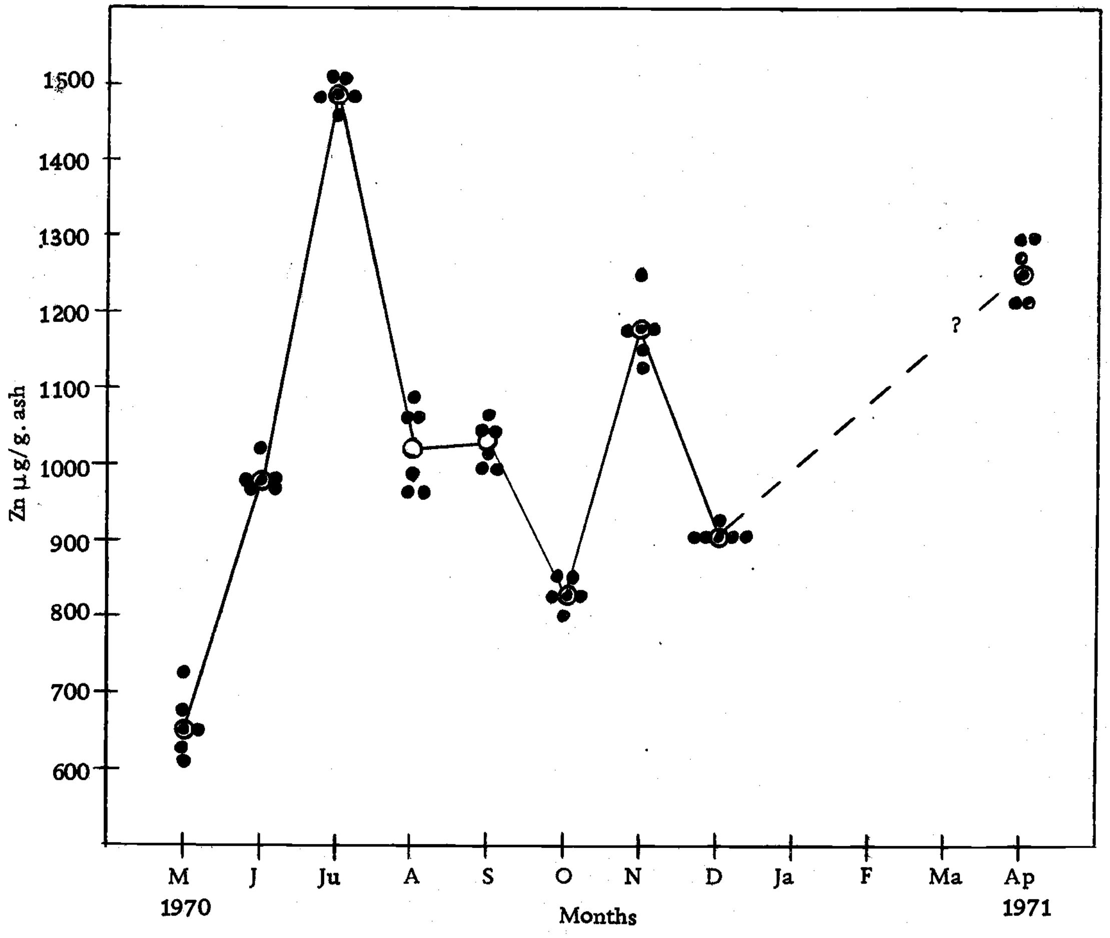

Figure 9. Zinc contents in portion A (foot, neck mantle, adductor muscle and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March *

* ● represents one measurement; ○ represents mean of six measurements on one sample

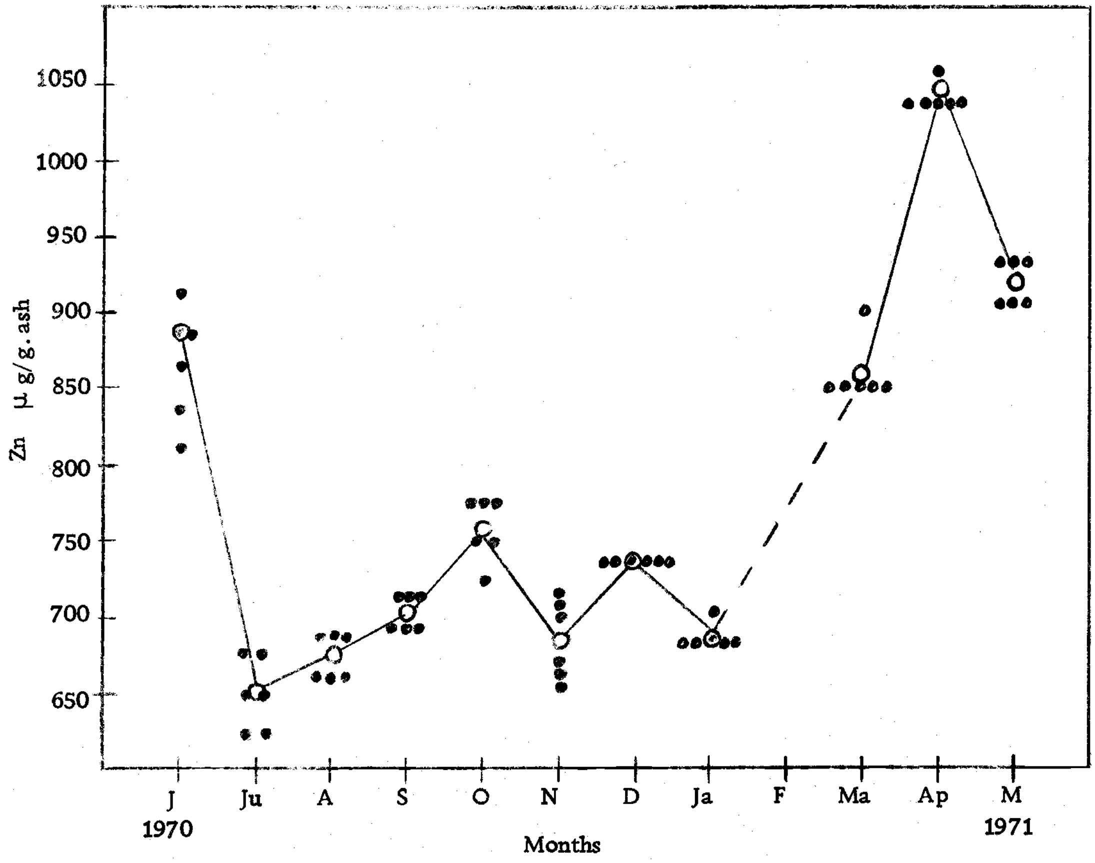

Figure 10. Zinc contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Long Beach (µg/g. ash). None collected in Feb.*

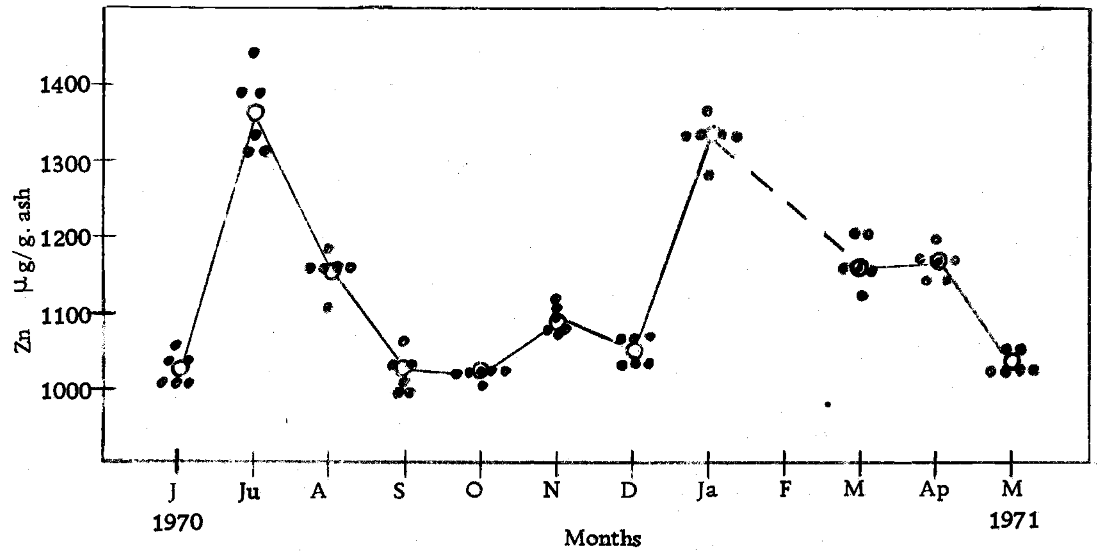

Figure 11. Zinc contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Long Beach (µg/g. ash). Non collected in February *

* ● represents one measurement; ○ represents mean of six measurements on one sample

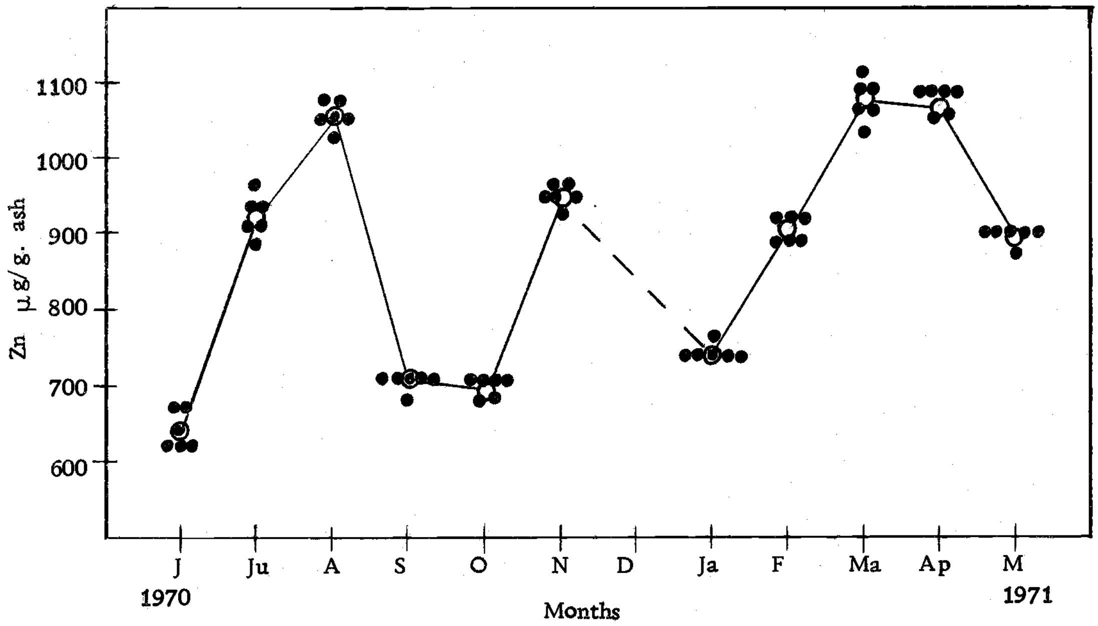

Figure 12. Zinc contents in portion B (gills, liver, digestive system and reproductive tissue) by month at ~~Roosevelt~~ Beach (µg/g. ash). None collected in December*

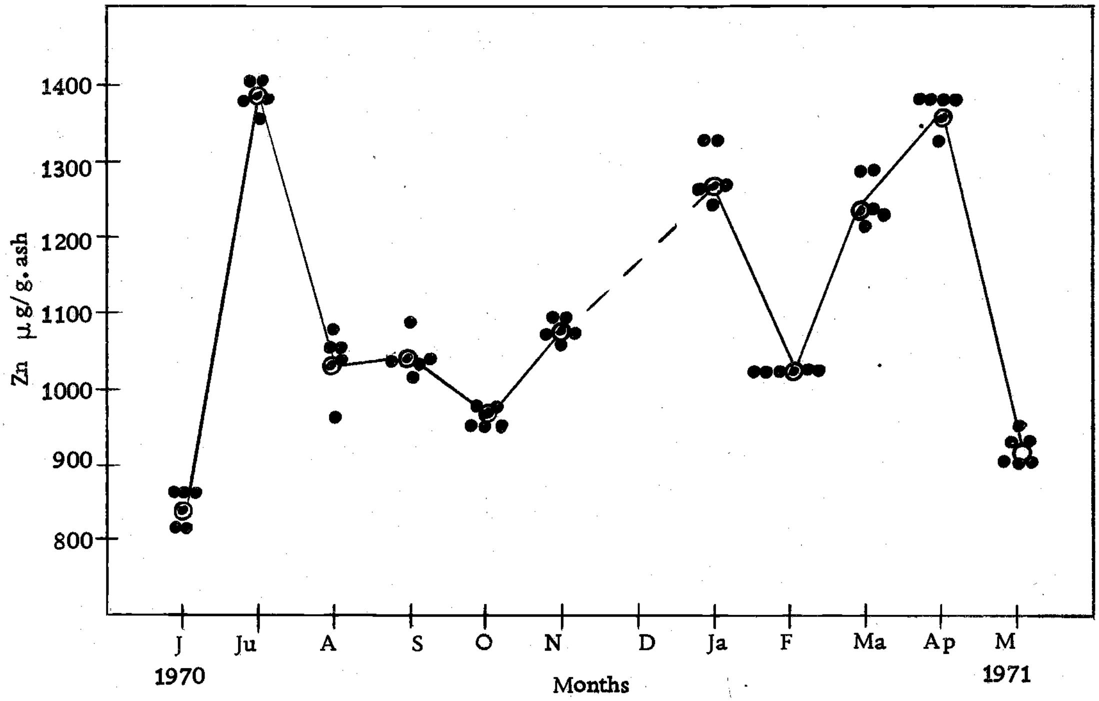

Figure 13. Zinc contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Roosevelt Beach (µg/g. ash). None collected in December *

* ● represents one measurement; ○ represents mean of six measurements on one sample

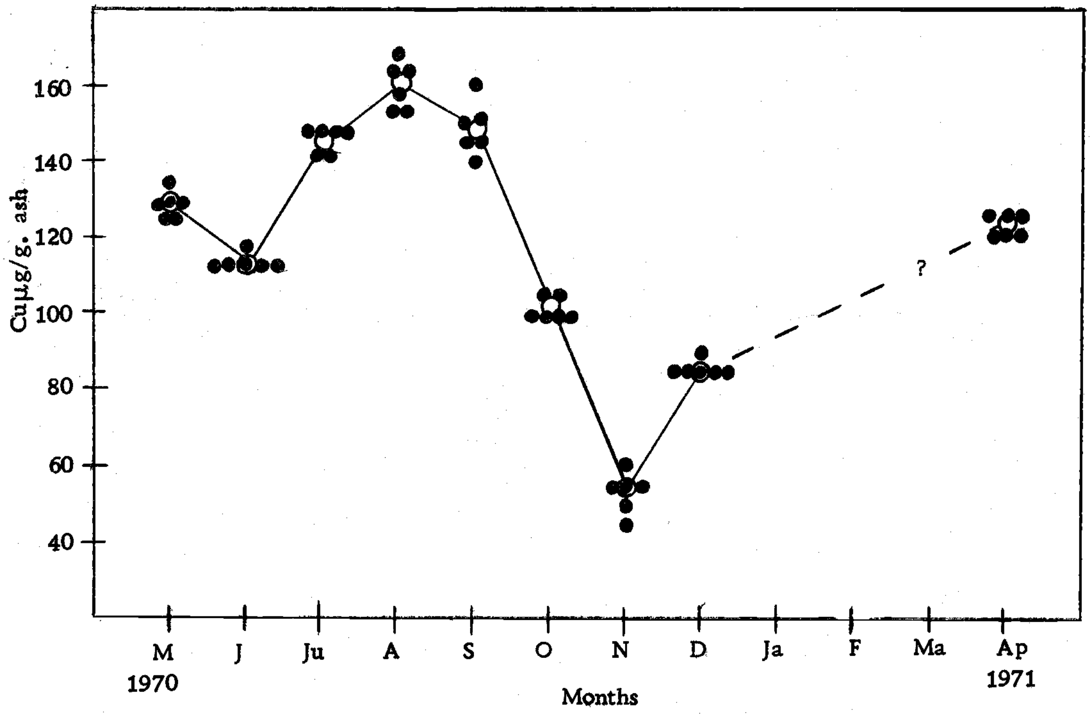

Figure 14. Copper contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March*

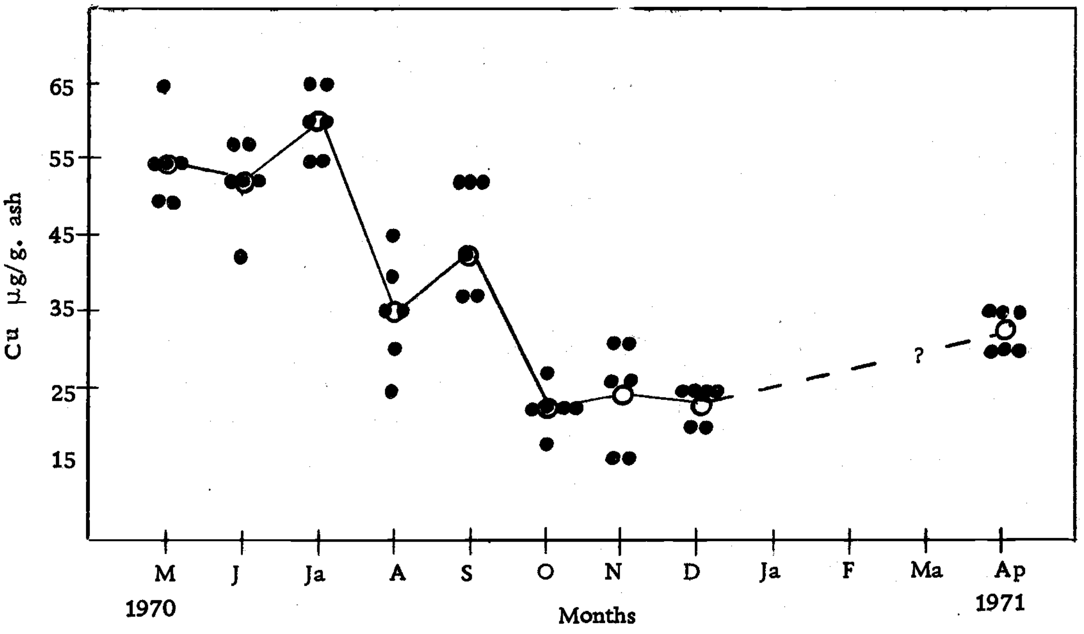

Figure 15. Copper contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March*

* ● represents one measurement; ○ represents mean of six measurements on one sample

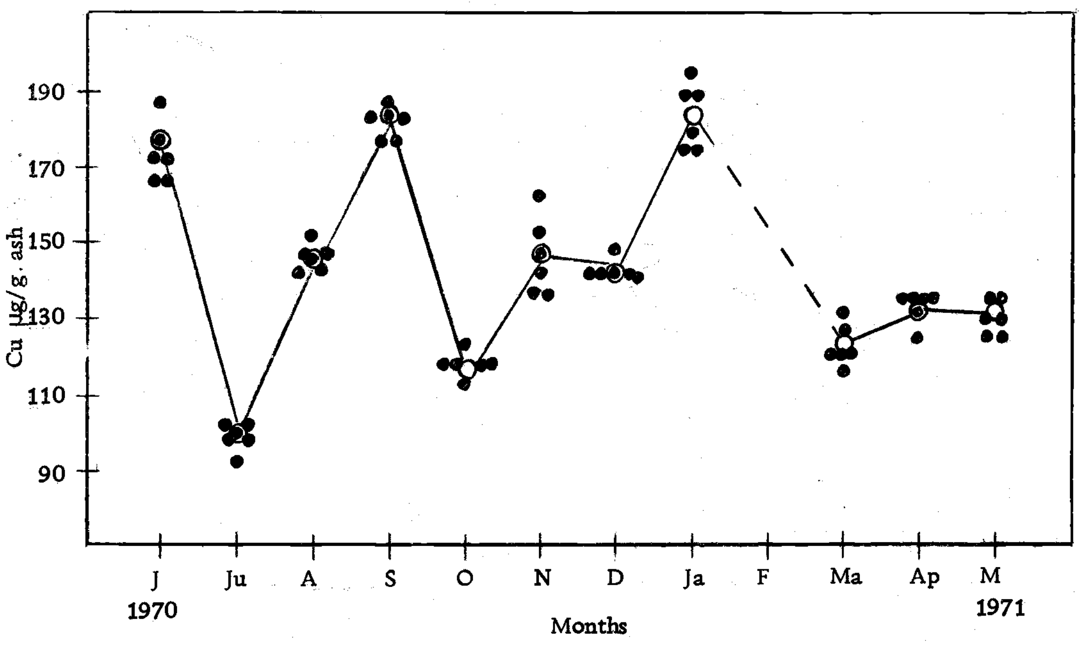

Figure 16. Copper contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Long Beach (µg/g. ash). None collected in February*

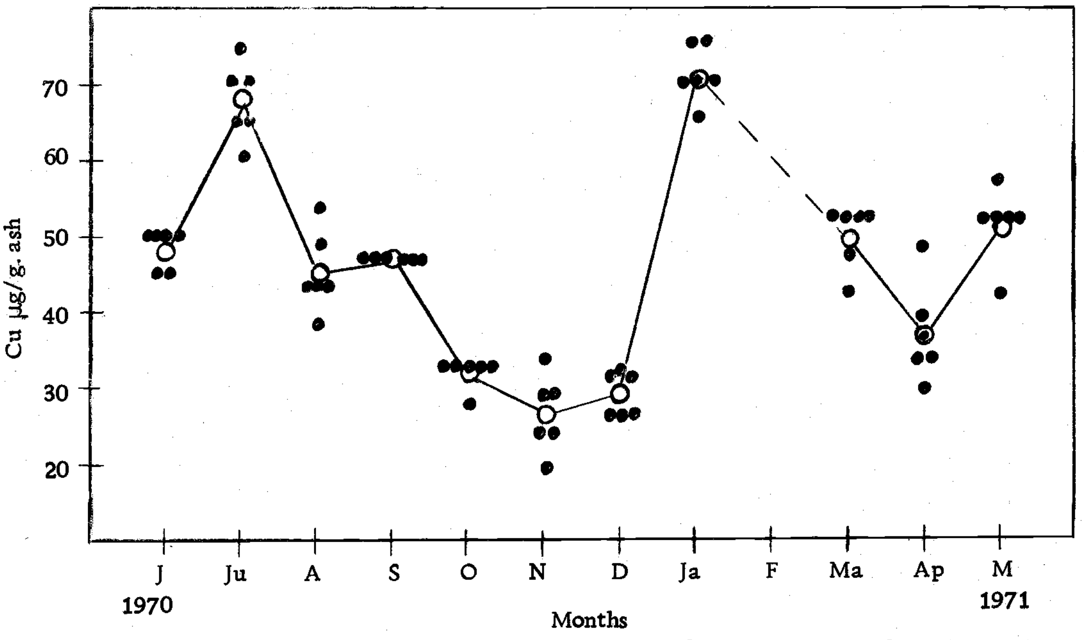

Figure 17. Copper contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Long Beach (µg/g. ash). None collected in February*

* ● represents one measurement; ○ represents mean of six measurements on one sample

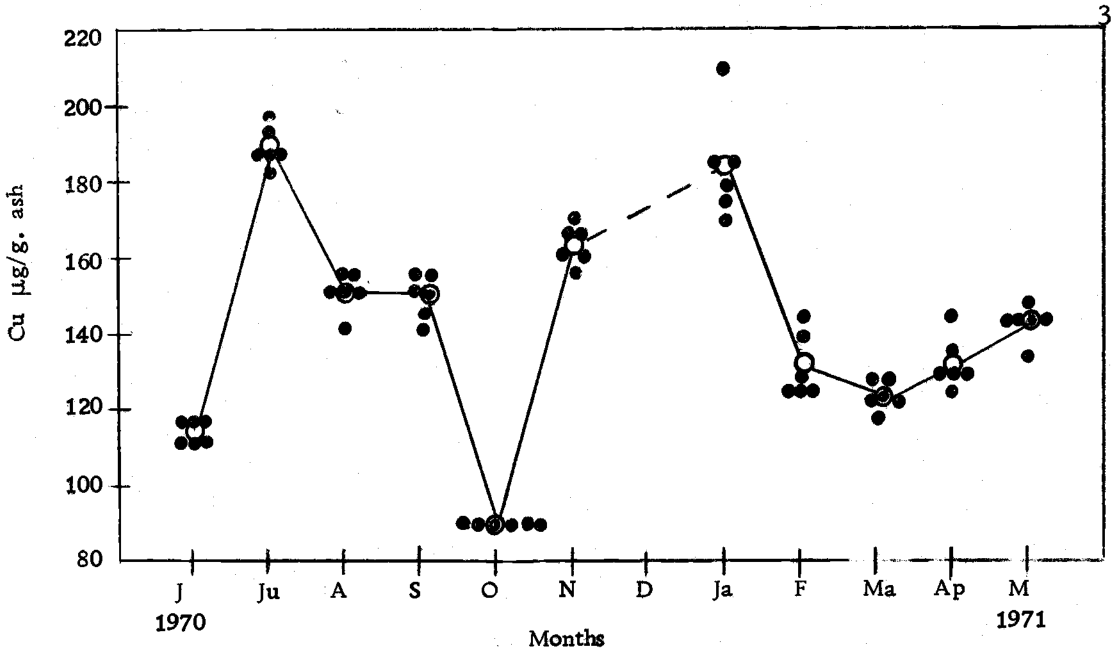

Figure 18. Copper contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Roosevelt Beach (µg/g. ash). None collected in December *

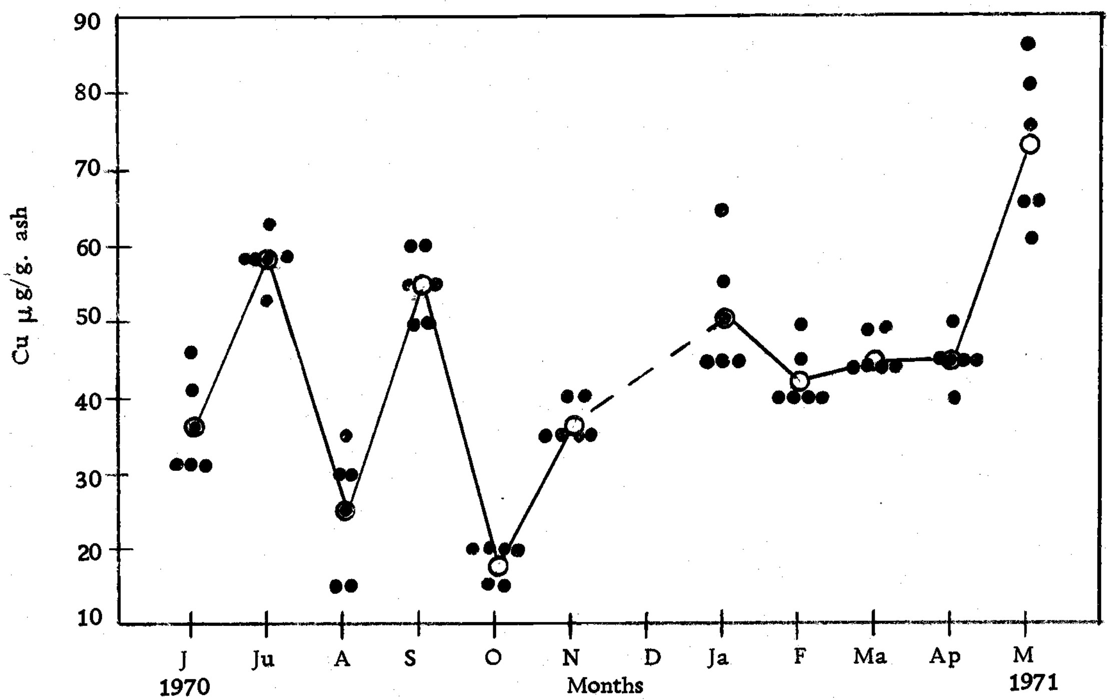

Figure 19. Copper contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Roosevelt Beach (µg/g. ash). None collected in December*

* ● represents one measurement; ○ represents mean of six measurements on one sample.

portion A at all three sites a very definite trend of increasing concentration in the Spring and Summer is indicated by Figures 21, 23 and 25. While the manganese concentration in portion B indicated in Figures 20, 22 and 24 generally conforms to this pattern it is much less evident.

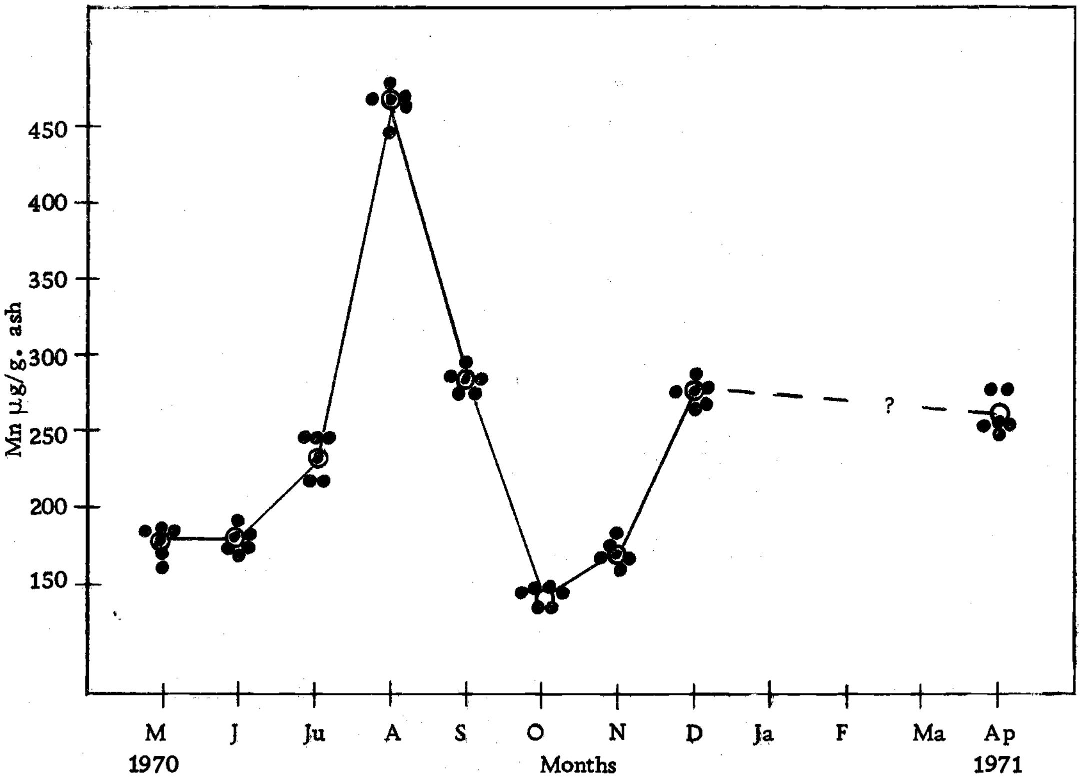

Figure 20. Manganese contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March*

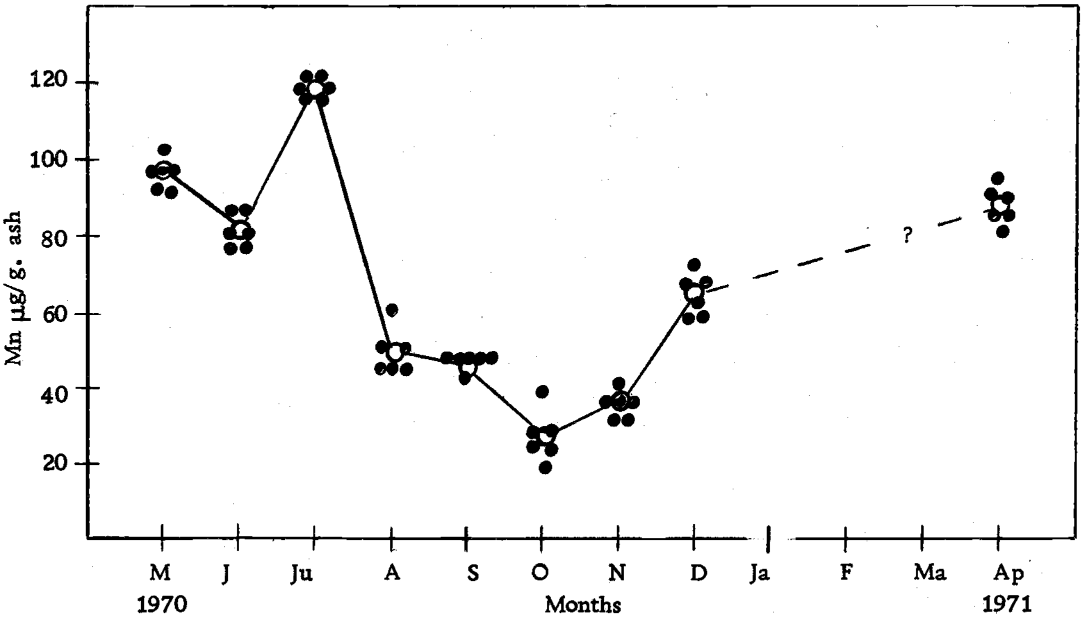

Figure 21. Manganese contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Sunset Beach (µg/g. ash). None collected Jan-March*

* ● represents one measurement; ○ represents mean of six measurements on one sample

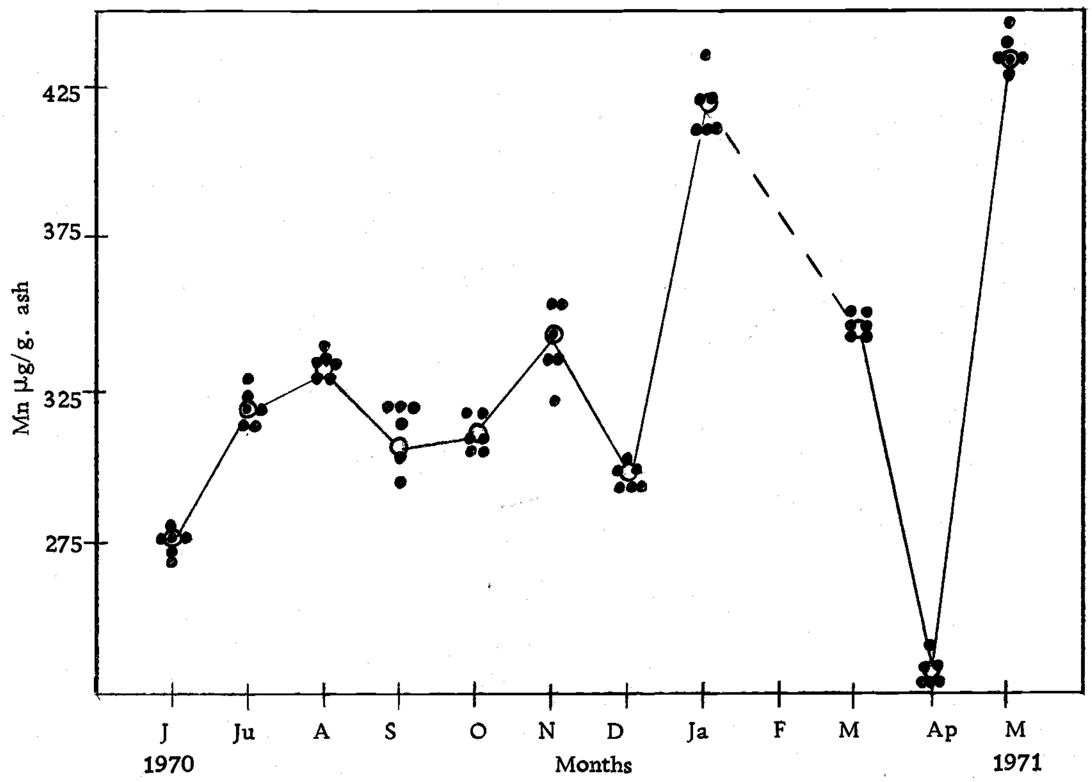

Figure 22. Manganese contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Long Beach (µg/g. ash). None collected in February*

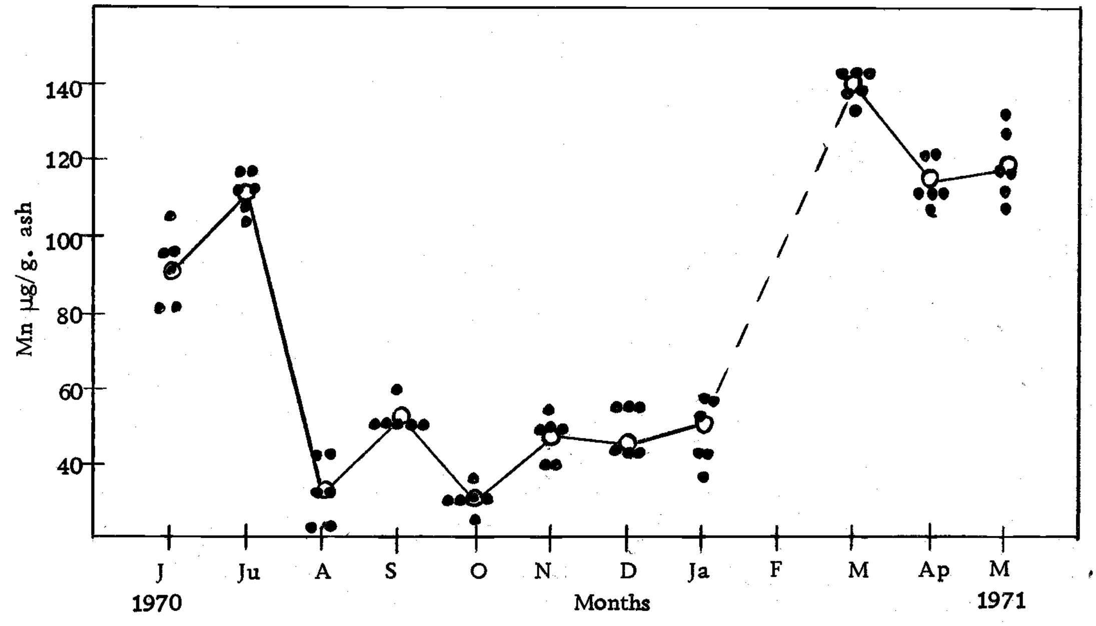

Figure 23. Manganese contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Long Beach (µg/g. ash). None collected in February*

* ● represents one measurement; ○ represents mean of six measurements on one sample

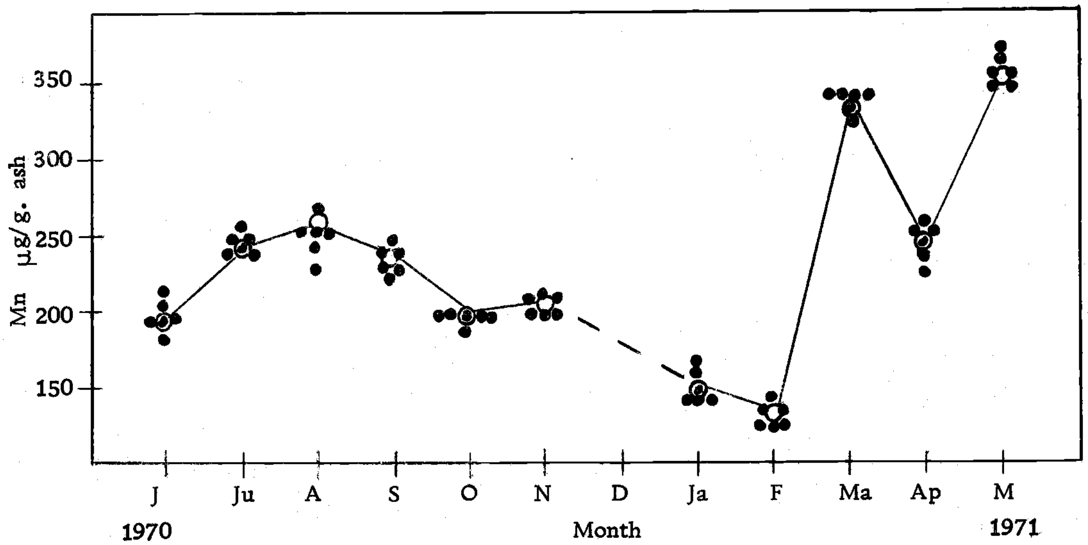

Figure 24. Manganese contents in portion B (gills, liver, digestive system and reproductive tissue) by month at Roosevelt Beach (µ g/g. ash). None collected in December*

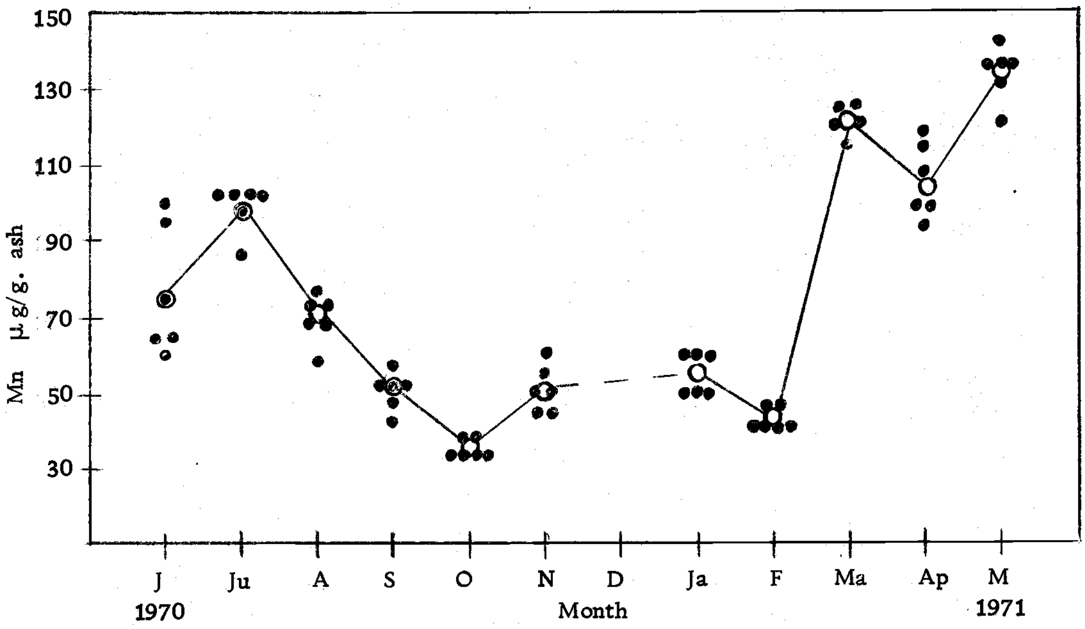

Figure 25. Manganese contents in portion A (foot, neck, mantle, adductor muscle and reproductive tissue) by month at Roosevelt Beach (µg/g. ash). None collected in December*

* ● represents one measurement; ○ represents mean of six measurements on one sample

DISCUSSION

In the preliminary study a statistically significant difference in the concentration of all three elements was observed among individuals. As indicated in the introduction there are numerous biological and physical factors that can affect the ability of an organism to concentrate trace metals and in turn produce variation among individuals. While many of these factors are known, at the present time there is a great deal that is not understood about the variation among individuals.

Based on the degree of variation present among the individuals in the preliminary study, it was estimated that a monthly composite sample size of at least 50 clams is required to detect a true 10% difference between month or site mean values.

From an examination of the trace element content in portions A and B it is evident that a significant difference between the two groups of tissue exists. The copper and manganese content being consistently higher in portion B while the reverse condition generally existing for zinc plus the fact that zinc was accumulated to a much greater extent suggests that the zinc requirement of S. patula is much greater than the requirement for copper or manganese.

While the variation between sites was in most instances statistically significant, based on the degree of individual variation, in the author's opinion further investigation is required before one may conclude that the difference between sites actually reflects a difference due to geographic location.

As indicated in the introduction zinc, copper and manganese are essential requirements for many of the reactions involved in metabolism. Table 7 indicates some of the enzymes requiring these trace metals. It is conceivable that a correlation exists between the trace metal content of an animal and its metabolic activity. However, before any attempt to correlate trace metal content with changes in metabolic activity it is important to recognize that the trace metals in question and the metabolic enzymes they affect are only part of a complex system responding to numerous physical and biological factors.

As indicated in the introduction prior investigations (Tegelberg, 1964 and Tegelberg and McGoon, 1969) suggest that the razor clam undergoes most of its growth and reproductive activity in Spring and early Summer which would indicate a change in metabolic activity. It is possible that the increased concentration noted for all three elements in Spring and Summer reflects an increased requirement resulting from these changes in metabolic activity. However it should be noted that since the monthly sample size was insufficient to reduce the effect of individual variation, the monthly variation could be reflecting the wide variability among individuals rather than a change in requirement. Continued monthly sampling and individual tissue analysis are required before any definite conclusions may be drawn.

Table 7. Some metabolic enzymes associated with zinc, copper and manganese.

Zn^{2+}

- Carbonic anhydrase
- Carboxypeptidase
- Protease
- Alkaline phosphatase
- Ethanol dehydrogenase (liver)
- Ethanol dehydrogenase (yeast)
- Lactic dehydrogenase
- Glutamic dehydrogenase
- D-Lactic cytochrome reductase

Cu^{2+}

- Tyrosinase
- Cytochrome oxidase
- Phenol oxidase
- Uricase
- Laccase
- Monoamine oxidase
- Ascorbic acid oxidase
- Ceruloplasmin
- Galactose oxidase

Mn^{2+}

- NADH nitroreductase
- Nitrite reductase
- Arginase
- Phosphotranases
- Isocitrate dehydrogenase

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APPENDIX

Appendix Table 1. Zinc, copper and manganese data in portions A and B by month at Sunset Beach (µg/g. ash)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
May 1970	625	857.5	64	129.5	92.5	184
	725	822.5	54	129.5	92.5	184
	600	882.5	54	129.5	97.5	184
	650	832.5	54	124.5	97.5	179
	650	807.5	49	124.5	97.5	169
	675	932.5	49	134.5	102.5	164
June	1020	770	57.5	112.5	87.5	170
	970	745	57.5	112.5	87.5	180
	970	745	52.5	112.5	82.5	180
	970	745	52.5	112.5	82.5	185
	970	720	52.5	112.5	77.5	175
	970	720	42.5	117.5	77.5	175
July	1482.5	862.5	60	147	118.5	235
	1482.5	837.5	60	147	118.5	235
	1482.5	912.5	65	147	119.5	235
	1507.5	912.5	65	147	119.5	230
	1507.5	887.5	55	142	120.5	220
	1457.5	887.5	55	142	120.5	220
August	987.5	912.5	25	163.5	60	447
	962.5	912.5	30	163.5	50	472
	962.5	912.5	35	153.5	50	467
	1062.5	912.5	35	153.5	45	467
	1062.5	887.5	40	158.5	45	467
	1087.5	937.5	45	168.5	45	467
September	992.5	692.5	37.5	145	43.5	280
	992.5	692.5	37.5	145	48.5	280
	1067.5	692.5	42.5	150	48.5	280
	1042.5	717.5	47.5	150	48.5	275
	1042.5	717.5	47.5	140	48.5	275
	1017.5	717.5	47.5	160	48.5	285
October	825	700	22.5	100	29	145
	825	700	22.5	100	29	145
	825	700	22.5	100	24	145
	800	725	22.5	100	24	145
	850	725	17.5	105	39	140
	850	725	27.5	105	19	140
November	1125	605	26	55	42.5	174
	1175	615	26	60	37.5	169
	1175	622	16	55	37.5	169
	1175	622	16	55	37.5	169
	1150	625	31	50	32.5	179
	1250	612	31	45	32.5	164

Appendix Table 1 (Continued)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
December 1970	925	550	20	90	68.5	281.5
	900	550	20	85	68.5	268.5
	900	550	25	85	58.5	276.5
	900	550	25	85	58.5	276.5
	900	575	25	85	73.5	271.5
	900	575	25	85	63.5	271.5
January	*NC	NC	NC	NC	NC	NC
February	NC	NC	NC	NC	NC	NC
March	NC	NC	NC	NC	NC	NC
April	1220	1007.5	30	126.5	85	255
	1220	1007.5	30	126.5	85	255
	1245	1007.5	30	126.5	90	255
	1295	1032.5	35	121.5	90	275
	1295	1032.5	35	121.5	80	275
	1270	982.5	35	121.5	95	250

*None Collected

Appendix Table 2. Zinc, copper and manganese data in portions A and B by month at Long Beach (μg/g. ash)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
June 1970	1040	862.5	50	177	105	277.5
	1040	812.5	50	167	95	277.5
	1065	837.5	50	162	95	277.5
	1015	887.5	50	162	90	282.5
	1015	887.5	45	157	80	272.5
	1015	912.5	45	157	80	267.5
July	1392.5	675	75	83.5	117.5	313.5
	1392.5	675	70	93.5	117.5	313.5
	1317.5	650	70	93.5	112.5	318.5
	1342.5	650	65	93.5	112.5	318.5
	1417.5	625	65	88.5	107.5	323.5
	1442.5	625	60	88.5	102.5	328.5
August	1162.5	687.5	43.5	132	42	328.5
	1162.5	687.5	43.5	132	42	328.5
	1162.5	687.5	43.5	132	32	333.5
	1162.5	662.5	38.5	137	32	333.5
	1187.5	662.5	48.5	137	22	333.5
	1112.5	662.5	53.5	142	22	338.5
September	992.5	692.5	47	177	50	313.5
	992.5	692.5	47	172	50	293.5
	1067.5	692.5	47	172	50	303.5
	1042.5	717.5	47	172	50	308.5
	1042.5	717.5	47	167	60	308.5
	1017.5	717.5	47	167	50	308.5
October	1025	775	32	113.5	30	305
	1025	775	32	108.5	30	305
	1025	775	32	108.5	30	310
	1025	750	32	108.5	30	310
	1025	750	32	108.5	35	315
	1000	725	27	103.5	25	315
November	1087.5	660	24	142.5	49	355
	1087.5	665	24	132.5	49	355
	1102.5	670	19	127.5	49	335
	1097.5	712.5	29	127.5	54	335
	1105.0	707.5	29	137.5	39	345
	1082.5	702.5	34	152.5	39	320
December	1037.5	737.5	26.5	132.5	42.5	293.5
	1037.5	737.5	26.5	132.5	42.5	293.5
	1037.5	737.5	26.5	132.5	42.5	293.5
	1062.5	737.5	31.5	132.5	47.5	298.5
	1062.5	737.5	31.5	132.5	47.5	298.5
	1062.5	737.5	31.5	137.5	47.5	303.5

Appendix Table 2 (Continued)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
January 1971	1292.5	682.5	70	179	52.5	420
	1342.5	682.5	70	179	42.5	420
	1342.5	682.5	70	184	42.5	410
	1342.5	682.5	75	169	57.5	410
	1342.5	682.5	75	164	57.5	410
	1367.5	707.5	65	164	47.5	435
February	*NC	NC	NC	NC	NC	NC
March	1157.5	850	47.5	106.5	137.5	341.5
	1157.5	850	52.5	111.5	137.5	341.5
	1157.5	850	52.5	111.5	142.5	346.5
	1132.5	850	52.5	111.5	142.5	346.5
	1207.5	850	52.5	116.5	142.5	351.5
	1207.5	900	42.5	121.5	132.5	351.5
April	1195	1037.5	29	125	111.5	323.5
	1145	1037.5	34	125	111.5	323.5
	1145	1037.5	34	125	111.5	227.5
	1170	1037.5	39	125	106.5	227.5
	1170	1037.5	39	120	121.5	227.5
	1170	1062.5	49	115	121.5	242.5
May	1025	907.5	57.5	115	106.5	432.5
	1025	907.5	52.5	115	116.5	432.5
	1025	907.5	52.5	120	116.5	432.5
	1025	932.5	52.5	120	111.5	427.5
	1050	932.5	52.5	125	131.5	437.5
	1050	957.5	42.5	135	126.5	442.5

*None Collected

Appendix Table 3. Zinc, copper and manganese data in portions A and B by month at Roosevelt Beach (µg/g. ash)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
June 1970	862.5	670	31.5	116.5	65	192.5
	862.5	670	31.5	116.5	65	192.5
	862.5	645	31.5	116.5	60	192.5
	837.5	620	36.5	111.5	75	202.5
	812.5	620	41.5	111.5	95	207.5
	912.5	620	46.5	111.5	100	177.5
July	1375	912.5	53.5	197	97	238.5
	1375	912.5	58.5	187	102	238.5
	1375	937.5	58.5	187	102	238.5
	1350	937.5	58.5	187	102	248.5
	1400	887.5	58.5	182	102	243.5
	1400	962.5	63.5	192	87	243.5
August	950	1025	35	140	73.5	252
	1025	1050	30	150	73.5	252
	1025	1050	30	150	78.5	252
	1050	1050	25	150	68.5	262
	1050	1075	15	155	68.5	237
	1075	1075	15	155	58.5	227
September	1035	710	55	140	42	235
	1035	710	55	145	52	235
	1035	710	60	150	52	240
	1010	710	60	150	52	230
	1085	685	50	155	47	230
	1035	710	50	155	57	220
October	950	682.5	20	88.5	39	192.5
	950	682.5	20	88.5	39	192.5
	950	707.5	20	88.5	34	192.5
	975	707.5	20	88.5	34	192.5
	975	707.5	15	88.5	34	192.5
	975	707.5	15	88.5	34	187.5
November	1081	945	35	160	45	205
	1077	945	35	160	45	205
	1075	942	35	165	50	205
	1085	932	35	165	50	200
	1070	960	40	170	60	200
	1067	960	40	155	55	200
December	*NC	NC	NC	NC	NC	NC
January 1971	1237.5	742.5	45	180	50	155
	1327.5	742.5	45	175	50	150
	1327.5	742.5	45	170	50	145
	1262.5	742.5	65	185	60	145
	1262.5	742.5	55	185	60	145
	1262.5	767.5	50	210	60	160

Appendix Table 3 (Continued)

	Zinc		Copper		Manganese	
	A	B	A	B	A	B
February 1971	1025	897	50	129	46.5	126
	1025	895	40	124	46.5	126
	1025	895	40	124	41.5	126
	1025	920	40	124	41.5	141
	1025	920	40	144	41.5	136.5
	1025	920	45	139	41.5	136.5
March	1237.5	1037.5	44	122.5	125	339
	1237.5	1062.5	44	122.5	115	339
	1237.5	1062.5	44	122.5	115	339
	1212.5	1112.5	44	117.5	120	339
	1287.5	1087.5	49	127.5	120	329
	1287.5	1087.5	49	127.5	120	334
April	1350	1082.5	50	124	99	250
	1325	1082.5	40	129	99	250
	1375	1082.5	45	129	94	245
	1375	1082.5	45	129	109	240
	1375	1057.5	45	134	114	235
	1375	1057.5	45	144	119	255
May	900	900	66.5	147.5	121	265
	900	900	66.5	142.5	131	355
	900	900	61.5	142.5	136	355
	925	900	76.5	142.5	136	350
	925	900	81.5	142.5	136	350
	950	875	86.5	132.5	141	360

*None Collected

Appendix Table 4. Ash and dry weights of monthly samples collected at Sunset Beach, Long Beach and Roosevelt Beach (gram wt.)

	Sunset Beach				Long Beach				Roosevelt Beach			
	ash		dry		ash		dry		ash		dry	
	A	B	A	B	A	B	A	B	A	B	A	B
May	8.14	2.36	72.34	18.81								
June	7.06	2.96	92.02	19.310	5.11	2.22	58.82	13.6	17.82	8.37	197.52	46.75
July	4.65	1.58	60.39	10.53	1.71	0.454	22.23	3.03	4.16	1.64	54.05	10.95
August	3.00	1.94	81.38	16.43	2.26	1.2	43.66	8.54	3.11	1.40	83.4	15.34
September	4.81	1.70	74.95	14.68	2.5	1.11	40.75	9.3	6.01	2.6	99.09	19.64
October	3.42	1.23	54.92	10.68	4.75	1.95	92.14	17.72	10.10	3.3	176.48	31.63
November	4.30	1.98	89.7	15.20	4.17	2.1	71.41	17.30	10.20	2.55	193.30	33.44
December	6.10	2.24	84.22	17.25	5.98	2.47	97.64	18.31	NC		NC	
January 1971 *	NC		NC		4.23	2.0	87.3	17.86	10.26	3.24	174.76	31.44
February	NC		NC		NC		NC		8.75	3.41	142.58	37.97
March	NC		NC		7.7	1.63	105.00	36.08	10.25	4.93	121.25	50.26
April	5.80	2.3	77.84	15.23	6.7	2.85	96.99	28.51	11.01	5.77	168.21	51.67
May					7.54	4.2	99.73	26.63	12.12	5.22	148.41	37.06

Nine collected at Long Beach and Roosevelt Beach during July through September.

*None collected