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The Dynamics of Metals in the American Oyster, *Crassostrea virginica*. I. Seasonal Effects[1]

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ABSTRACT: The seasonal dynamics of Mn, Fe, Zn, Cu and Cd were investigated in a genetically similar population of hatchery-reared American oysters, *Crassostrea virginica*, maintained in plastic trays in the Rhode River, a tributary of the Chesapeake Bay. Samples were collected monthly from September, 1971 through May, 1973. Annual cycles resulting in the turnover of large portions of the body burden were observed for all metals studied. Subtly different patterns of metal dynamics were observed as a result of the reduction in biological variations realized by employing genetically similar oysters. Metals are grouped into two classes according to their dynamics; (1) Mn and Fe concentrations in soft tissues are significantly correlated with shell deposition. A high rate of Mn turnover in soft tissues (approximately 2 times the body burden per day) occurs during the shell growth season. (2) Zn and Cu concentrations are not correlated with shell growth. Zn and Cu body burdens exhibit a gradual increase during the spring and early summer followed by a rapid loss during August–September in which 33% of the Zn and 50% of the Cu is lost in less than 4 weeks. Cd behavior is similar to Zn and Cu with a 50% reduction in body burden during an 11 week period between July and October.

Introduction

The ability of the American oyster, *Crassostrea virginica*, to accumulate high concentrations of metals in its soft tissues is well documented (Hiltner and Wickman 1919; Bodansky 1920; Rose and Bodansky 1920; Severy 1922; Coulson *et al.* 1932; Chipman *et al.* 1958; McFarren *et al.* 1961; Galtsoff 1964; Brooks and Rumsby 1965; Pringle *et al.* 1968; Kopfler and Mayer 1969; Shuster and Pringle 1969; Roosenburg 1969; Wolfe 1970a; Huggett *et al.* 1971; and Windom and Smith 1972). From a public health point of view, the accumulation of metals in human food resources should be considered with some degree of concern because of the implications of certain metals in chronic and acute health problems. For this reason, an understanding of the environmental and biological factors which determine the dynamics of metals in the soft tissues of oysters must be developed, particularly with respect to the impact of human activities on the basic processes involved. This paper is the first of a series of papers directed toward this goal. Here we consider the baseline seasonal dynamics of Mn, Fe, Zn, Cu, and Cd in *C. virginica* under natural conditions.

Seasonal fluctuations in the metal composition of oysters have been suggested by several researchers (Galtsoff 1942, 1964; McFarren *et al.* 1961; Pringle *et al.* 1968; Kopfler and Mayer 1969; and Roosenburg 1969). Galtsoff (1964) has carried out detailed investigations specifically designed to study this phenomenon. His data clearly show a seasonal pattern for the concentrations of Mn, Fe, Cu and Zn in soft tissues: elevated levels of metals in the summer are followed by depressed levels in the winter. Our data

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verify this general pattern; however, the use of a unique experimental design reduces the biological variability involved, thus permitting the observation of subtle variations in the seasonal cycle of different metals.

Methods

Most research in the past designed to investigate metal metabolism in oysters has used natural populations of animals. This has led to limitations in the interpretation of results due to biological variability inherent in these populations. The main sources of this variability are genetic polymorphism exhibited by natural populations and the difficulty in identifying homogeneous age classes, i.e. animals with identical life histories.

In order to minimize biological variability, hatchery-reared oysters were employed in this investigation. These oysters were derived from a single parent pair, thus increasing genetic homogeneity. Furthermore, this genetically similar oyster population represents a homogeneous age class since the individual oysters were reared under identical conditions. By employing such an oyster population in these studies, sequential sampling on a monthly basis provided a true temporal picture of the dynamics of metal concentrations in the soft tissues. In addition, the total tissue body burden could be calculated (metal concentration times soft tissue weight) and observed in temporal sequence in order to determine whether observed fluctuations in metal concentrations were due to net metal flux into or out of the soft tissues or whether these fluctuations were due to changes in body weight.

Approximately 500 genetically similar oysters were placed in plastic trays and suspended from the pier at the Smithsonian Chesapeake Bay Center for Environmental Studies (CBCES) in the Rhode River, a small (3 × 1 km), unpolluted tributary of the Chesapeake Bay, on 28 September, 1971. The oysters, whose parents were collected from the same area, were 14 months old having a mean height of 36 ± 1 mm and a mean soft tissue dry weight of 0.123 ± 0.019 gm. All data are reported and graphed as the mean ± the standard error of the mean based on a sample of ten individuals.

Samples of 10 oysters were collected at monthly intervals from September, 1971 to May, 1973 and individual measurements were made of shell dimensions (height and width), wet weight of drained soft tissues, dry weight of soft tissues and Mn, Fe, Zn, Cu and Cd concentrations of soft tissues. Two groups of 5 oyster shells each, collected 28 March and 7 June, 1972, were analyzed individually for Mn, Fe, Zn, Cu and Cd. Three samples of bottom sediments at the CBCES pier were collected and analyzed for Mn, Fe, Cu, Zn, Cd, Co, Cr, Ni and Pb in order to verify the natural conditions of the environment.

For each of the 10 oysters constituting a monthly sample the following preparation was carried out. Each oyster was thoroughly washed and shucked. The entire soft body tissue was removed, allowed to drain for 5 minutes and then placed in a pre-weighed plastic container and sealed. Wet weight was determined and the sample frozen. The samples were freeze-dried for 48 hours and a dry weight determined. Soft tissues were digested in concentrated nitric acid plus 30% hydrogen peroxide (2:1, HNO_3:H_2O_2). Metal analyses for Mn, Fe, Zn, Cu and Cd were performed on a Perkin-Elmer (Model 303) atomic absorption spectrophotometer. Concentrations are expressed on a dry weight basis to reduce errors associated with random retention of body liquors. An approximate conversion from dry weight concentration to wet weight concentration for soft tissues requires dividing the dry weight concentration by 5.0 (actual ratios of wet weight: dry weight ranged from 4.74 to 5.51).

Oyster shells were crushed in a porcelain mortar and pestle, mixed and oven dried before an aliquot (1 gm) was taken and digested in the nitric acid-hydrogen peroxide mixture. Because of the high salts content of the digested shell matrix a background non-atomic absorption correction was employed using a deuterium lamp.

Metal analyses were performed on samples of surface sediments (top 5 cm) which were collected by an Eckman dredge, placed in plastic bags, and transported to the laboratory in ice. Visible debris was removed by hand. Approximately 200 ml of sediment per sample was vacuum-filtered to remove interstitial water and oven dried at 60 C to

constant weight. The dry sediment was then sieved through an 0.8 mm plastic screen to remove large pieces of organic matter and coarse sand. A 1 gm aliquot per sample was leached in hot, concentrated nitric acid for 4 hours, filtered through a pre-cleaned glass fiber filter and analyzed by atomic absorption spectrophotometry. Results are reported on a dry weight basis.

Physical data, salinity and temperature, were taken on a Honeywell Data Acquisition System 200 continuously recording monitor located at the CBCES pier approximately 30 feet from the oyster trays.

Results

The growth of oyster soft tissue, as measured by dry weight, for September 1971 to May 1973 is presented in Fig. 1. The pattern of growth is typical for the oyster (Galtsoff 1964): rapid growth during gonadal build up in the spring, loss of body weight during spawning, a second period of rapid growth in the fall when glycogen is being stored, followed by depletion of glycogen stores during the winter. Soft tissue growth was not correlated with salinity for the range of salinities observed in the Rhode River (3 o/oo–13 o/oo). Little growth occurred when the temperature was below 10 C or above 25 C. Overall, net growth resulted in a five-fold increase in total soft tissue solids during the 20 months of observation.

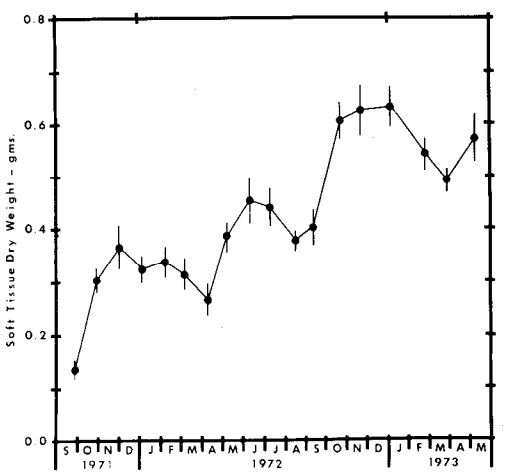

FIG. 1. Soft tissue dry weight of genetically similar oysters; mean ± standard error of the mean for each 10 oyster sample.

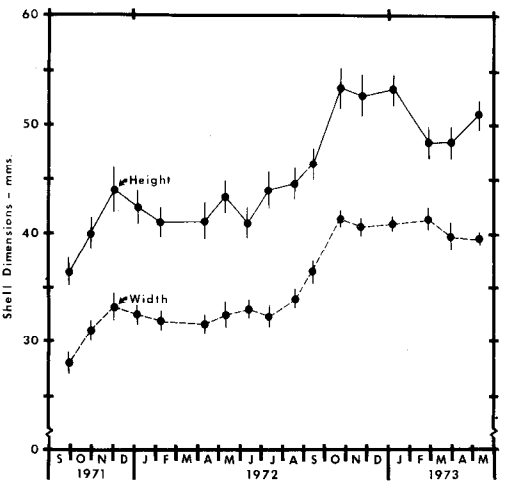

FIG. 2. Shell dimensions (height and width) of genetically similar oysters; mean ± standard error of the mean for each sample of 10 oysters.

Fig. 2 gives shell dimensions. In contrast to soft tissue growth, shell growth exhibited one extended growth period in 1972, July–November, in accordance with the observations of others (Galtsoff 1964; Quayle 1969). This shell growth season was significantly longer than either of the soft tissue growth periods and occurred during the hottest part of the year with water temperatures greater than 20 C. Shell dimensions tended to show a slight reduction during the winter. Net growth for the observation period was: height—15.5 mm and width—12.0 mm. During the shell growing season of 1972, between June and November, average shell weight increased by 5.94 gm.

The concentrations of Mn, Fe, Zn, Cu and Cd in oyster soft tissues are presented in Fig. 3 A-C. Each figure gives the metal concentration in soft tissues on a dry weight basis. The order of metal concentrations in soft tissues is Zn >> Fe > Cu > Mn > Cd. Seasonal fluctuations in metal concentrations are clearly evident, with elevated levels during the summer months most prominent. Zn, Cu, and Cd reach their peak summer concentrations in early August, then gradually fall to significantly lower winter levels by mid-October. Mn and Fe concentrations do not attain their peak levels until the first of October (± 2 weeks), then decrease to winter levels by the end of November. The data for Mn, Fe, Zn and Cu concentrations are in general agree-

ment with previous data (Galtsoff 1964), but are smaller in magnitude. The patterns of metal kinetics for the two groups of metals, Zn, Cu and Cd on the one hand and Mn and Fe on the other, are quite similar, however

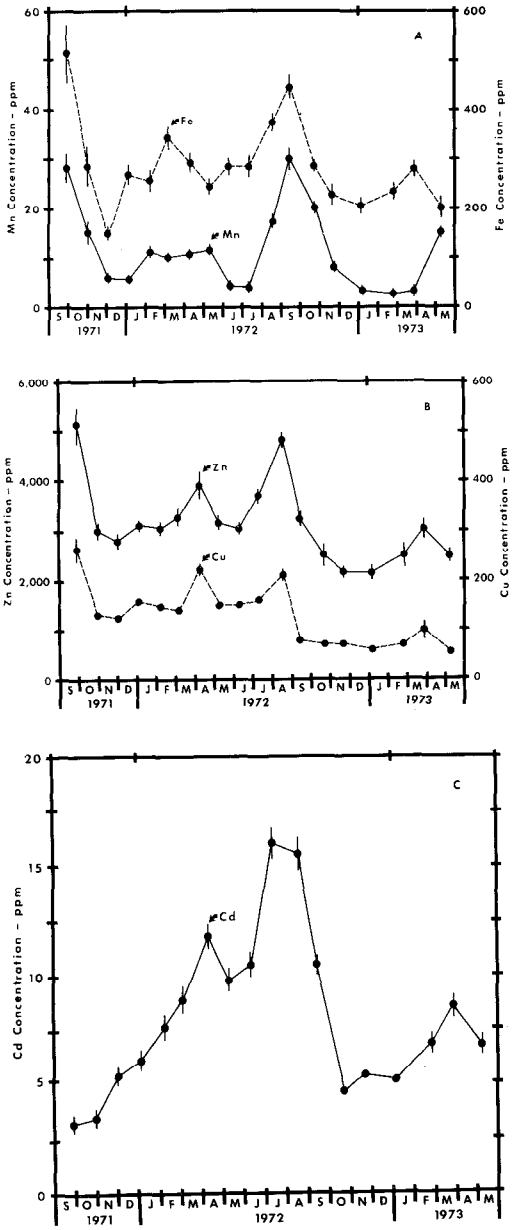

FIG. 3. (A, upper) Mn and Fe concentration, (B, middle) Zn and Cu concentration, and (C, lower) Cd concentration in whole soft tissues of genetically similar oysters; mean ± standard error of the mean for each sample of 10 oysters. No error bars indicate that SEM was less than size of symbols used.

there is a time lag of approximately 6 weeks between the two patterns with the Zn, Cu, Cd cycle preceeding the Mn, Fe cycle. The fact that the subtle difference between Mn-Fe and Zn-Cu-Cd concentrations in soft tissues can be distinguished here is a result of the reduction in biological variations realized by employing genetically similar oysters.

Fig. 4 A-C give the seasonal data for total body burdens (soft tissue concentration times soft tissue dry weight). Mn and Fe body burdens increase in the summer reaching a peak around the first of October (± 2 weeks) and then decrease to winter levels over a 10 week period at an average rate of 1 μg/week and 5 μg/week for Mn and Fe respectively. Zn and Cu body burdens increase linearly beginning in the spring, reaching a peak in early August and then rapidly decrease to winter levels in less than 4 weeks at an average rate of 120 μg/week and 10 μg/week for Zn and Cu, respectively. Cd increases throughout the winter and spring attaining its maximum level around the first of July, then gradually decreases over the next 11 weeks at an average rate of 0.35 μg/week.

The results for the analysis of shell samples are reported in Table 1. The order of metal concentrations in these shells is Mn >> Fe > Zn > Cu > Cd with the Mn concentration roughly two orders of magnitude greater than the other metals and the Cd concentration below the detection limit of the method of analysis. These data indicate no statistically significant seasonal variation in transitional metal composition of shell. Mn concentrations reported here are higher than reported elsewhere (Rucker and Valentine 1961; Windom and Smith 1972), but have been confirmed by analysis of other oyster shells from the same area (Frazier, unpublished data). Since Rucker and Valentine (1961) noted an inverse relationship between salinity and Mn concentration in shell, the low average salinity of the Rhode River could account for the high Mn concentrations observed. Fe and Zn concentrations are quite similar to those of Windom and Smith (1972), but Zn levels are low compared to Wolfe's data (1970a).

The metal composition of bottom sediments is given in Table 2. The order of metal concentrations is Fe >> Mn > Zn > Cr > Pb > Cu ≃ Ni > Co > Cd. The levels of all

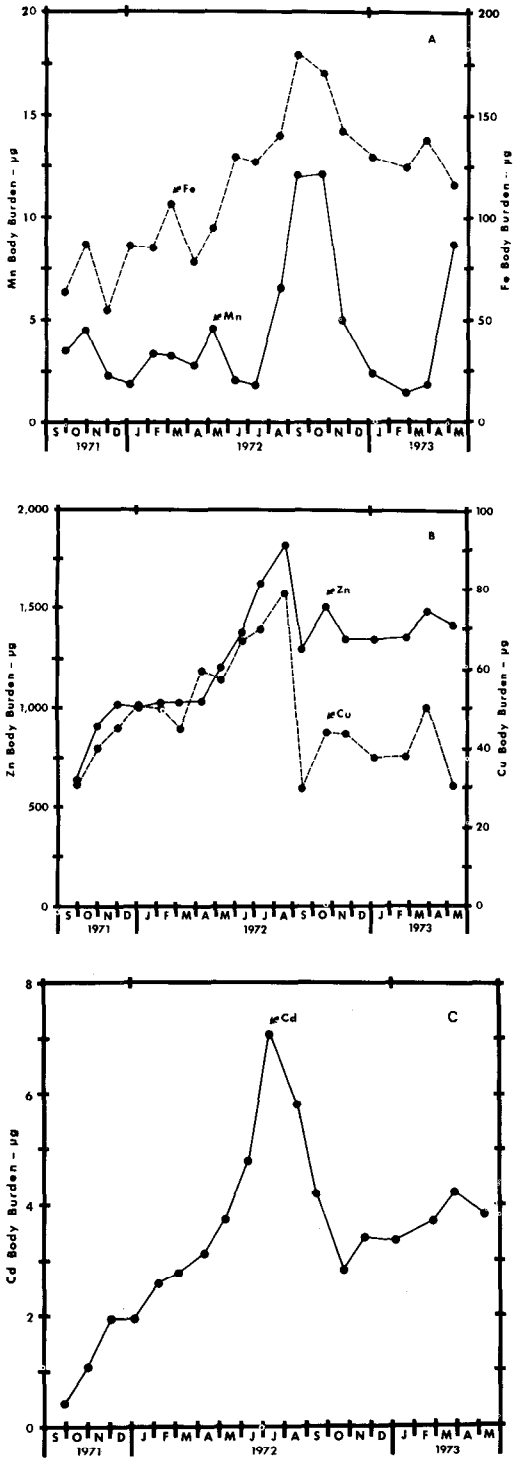

FIG. 4. (A, upper) Mn and Fe body burden, (B, middle) Zn and Cu body burden, and (C, lower) Cd body burden in whole soft tissues of genetically similar oysters.

TABLE 1. The metal composition of oyster shells collected 3/28/72 and 6/7/72 (Mean ± one standard deviation; n = 5).

	Concentration (µg/gm dry weight)				
Date	Mn	Fe	Zn	Cu	Cd
3/28/72	520 ±40	20 ±13	2.1 ±0.4	0.13 ±0.05	<0.1
6/7/73	490 ±80	18 ±8	3.0 ±1.6	0.19 ±0.09	<0.1
Average	505	19	2.5	0.16	<0.1

TABLE 2. Average metal composition of bottom sediments (top 5 cm) collected at the CBCES pier.

Metal	Concentration (µg/gm dry weight)
Mn	57.4
Fe	18,200
Cu	6.1
Zn	46.1
Cd	0.3
Co	2.7
Cr	31.9
Ni	6.1
Pb	11.1

metals in the sediments are characteristic of natural levels in the Chesapeake Bay area (Pheiffer 1972).

Discussion

Hypotheses suggested for the control of metal uptake by oysters may be grouped into four categories: (1) those related to shell growth and Ca metabolism (Galtsoff 1964; Wolfe 1970a, 1970b; and Coombs 1972), (2) those related to feeding (Hiltner and Wickman 1919; Korringa 1952; McFarren *et al.* 1961; Preson, 1971; and Kerfoot and Jacobs 1973), (3) those related to spawning (Galtsoff 1942, 1964), and (4) those related to physical chemistry of the metal either in the water or at the water-membrane interface (Rucker and Valentine 1961; Romeril 1971; Windom and Smith 1972; and Huggett *et al.* 1971).

In order to investigate the role of various biological processes in the metal dynamics of soft tissues, the following calculations were carried out: (1) the correlation between all possible pairs of soft tissue metal concentra-

tions, (2) the total amount of each metal deposited in the shell during the shell growth season, June to November, 1972, (3) the decrease in body burden of Zn, Cu and Cd during their periods of maximum loss, and (4) the correlation between each soft tissue metal concentration and the shell growth rate.

(1) The correlation coefficient (r) was calculated for each pair of soft tissue metal concentrations utilizing standard techniques, Table 3. A correlation coefficient greater than 0.50 is statistically significant at the 0.05 level. Mn is significantly correlated only with Fe and not with Zn, Cu or Cd. Fe is significantly correlated with Zn and Cd in addition to Mn. Zn, Cu and Cd are highly correlated with each other. Thus, in terms of correlation between soft tissue concentrations, the five metals fall into two groups; Mn on one hand and Zn, Cu and Cd on the other, with Fe intermediate, correlating with some members of both groups. These results are in general agreement with the data of Windom and Smith (1972) who calculated the correlations between Mn, Fe, Zn, Cu and Ag in soft tissues of natural populations of oysters. The only discrepancy being that they found no correlation between Fe and Zn, whereas, we found this correlation significant.

(2) The amount of metals deposited in the shell during the 1972 shell growing season is calculated as the product of the increase in shell weight during the interval (5.94 gm) and the concentration of the metal in the shell matrix (Table 1). This calculation and subsequent interpretation are based on the assumptions that: (a) the trace metal components of the shell matrix are derived from the soft tissues and not directly from the external environment by adsorption, and (b) trace metals are deposited in the shell matrix as a constant fraction of the Ca deposited. These assumptions imply that trace metals are homogeneously distributed in oyster shell and exhibit no seasonal fluctuations which is consistent with the data of Table 1. The magnitude of metal adsorption by means of ion-exchange at the external shell-water interface is not known, but the work of Romeril (1971) indicates that it may be significant. In that case, the amount of metals deposited in shell during the growing season will be overestimated. The results are given in the second column of Table 4 and can be compared to the observed range of body burdens for each metal.

TABLE 3. Correlation coefficients between oyster soft tissue metal concentrations.

	Fe	Zn	Cu	Cd
Mn	0.66*	0.29	0.01	0.18
Fe	—	0.63*	0.35	0.61*
Zn	—	—	0.87*	0.88*
Cu	—	—	—	0.71*

* Statistically significant at the 0.05 level.

TABLE 4. Range of body burdens, amount of metal deposited in shell during the 1972 shell growing season and the decrease in body burden for Cu, Zn and Cd during the 1972 shell growing season.

Metal	Range of Body Burden (μg)	Metal Deposited in Shell (μg)	Decrease in Body Burden (μg)
Mn	2–13	3,020	—
Fe	50–180	112	—
Zn	600–1800	15	500[1]
Cu	30–80	1	55[1]
Cd	0.5–7.0	<0.6	5.0[2]

[1] During August–September.
[2] During July–October.

The five metals can be divided into three groups on the basis of the relation between metal deposited in shell and average body burden. For Mn, the amount deposited in shell is in great excess of the body burden implying a rapid turnover of Mn in soft tissues during this period. For Fe the amount deposited in shell is on the same order of magnitude as the body burden. For Zn, Cu, and Cd the amount deposited in shell is much less than the body burden.

(3) The decrease in Zn, Cu, and Cd body burdens during their periods of rapid discharge are calculated directly from the data of Fig. 2. The results are given in the last column of Table 4. The quantity of Zn and Cu lost during a one month period from mid-August to mid-September constitutes a significant fraction of the entire body burden (33% and 50% respectively). Furthermore, the amount lost dur ⸗ this month is much greater than the amount deposited in the shell over a four month period. Similarly, the

amount of Cd lost during the July–October interval is 50% of the peak body burden and is large compared to the amount of Cd deposited in shell.

(4) The calculation of the correlation between soft tissue metal concentration and shell growth rate required some quantitative estimate of the shell growth rate. The best estimate of this process would be the rate of Ca deposition, which could be calculated from detailed data on shell weight as a function of time. This data was not collected in our original experiments so we shall use as a crude estimate of shell growth rate the rate of change of shell dimensions, in particular the height. The needed data can be computed from Fig. 2. In cases where the height decreases, which implies a negative shell growth, we set the growth rate equal to zero since recent data (Frazier, unpublished data) indicates that shell weight does not change during the winter even though shell dimensions decrease. When a standard correlation coefficient is calculated, Table 5, it is found that Mn and Fe are significantly correlated with shell growth rate while Zn, Cu and Cd are not.

To summarize these data, we can identify two fundamentally different patterns of behavior exemplified by Mn and Zn. Mn concentrations and body burden in soft tissue tends to be low during the winter and shows a general increase throughout the shell growth season. Using the data for the quantity of Mn deposited in the shell during the growing season (approximately 3000 μg) and the length of the shell growing season (approximately 150 days) we get an average of 20 μg of Mn deposited in the shell matrix per day compared to a body burden of 10 μg. The amount of Mn deposited in the shell daily is in great excess of the body burden implying a rapid turnover of Mn in soft tissues during this period. The increase in Mn body burden during the shell growing season is the result of the net difference between two large rates, the soft tissue uptake rate and the shell deposition rate. The concentration of Mn in soft tissue is significantly correlated with shell growth. These characteristics are consistent with the hypothesis which would relate the control of this metal with shell growth and Ca metabolism. The pattern could also be enhanced by a general increase in Mn uptake related to increased feeding when the water temperature is greater than 10 C. The role of Mn in spawning as suggested by Galtsoff (1942, 1964) can not be determined from our data, but it appears that the magnitude of Mn flux as a result of shell deposition far exceeds that involved in other processes. The elucidation of the control mechanisms for Mn dynamics in relation to shell deposition, feeding, and spawning requires further research.

TABLE 5. Correlation coefficients between oyster soft tissue metal concentration and shell growth rate as measured by rate of change in shell height.

Mn	Fe	Zn	Cu	Cd
0.76*	0.60*	0.20	−0.12	0.22

* Statistically significant at the 0.05 level.

The correlation of the behavior of Fe in soft tissues with shell deposition is not as clear cut as that of Mn, indicating that other processes are influencing its behavior. However, the general similarities of seasonal patterns among Mn and Fe, and the fact that they correlate with each other justified grouping these two metals together with respect to their regulations.

In contrast to Mn, Zn tends to remain constant during the winter, increase rapidly in early summer followed by a dramatic loss of a large fraction of the body during a one month period in the middle of the shell growing season, August–September. The total amount of Zn deposited in the shell is very small compared to the quantity of Zn lost from soft tissues and hence cannot account for the drop in body burden. The soft tissue Zn concentration is not correlated with shell growth. Since several zinc metalloenzymes are involved in shell mineralization, i.e. carbonic anhydrase and alkaline phosphatase (Galtsoff 1964), and furthermore, Zn metalloenzyme activity is correlated with total Zn concentration in other biological systems (Reinhold *et al.* 1970), it would be expected that Zn tissue concentrations should increase with increasing shell mineralization. However, here we find that Zn concentrations in soft tissues dramatically decrease prior to the period of maximum shell growth. This would indicate that some other biological process is controlling Zn tissue concentrations. Zn metabolism

has been related to gametogenesis in several higher species (Fischer *et al.* 1955; Millar *et al.* 1958). A more likely explanation of the behavior of Zn metabolism would be its control by gonadal development and spawning. Zn need not be incorporated directly into gametes, although this is not ruled out, but could be involved in general metabolic processes of gonadal tissues during gamete development. The temporal correlation between the reproductive cycle and Zn soft tissue dynamics is highly suggestive.

The rapid discharge of Zn during August-September is indicative of a short biological half life on the order of 40-50 days. Such a short half life appears to contradict the reported biological half life for Zn of 300 days (Seymour 1966) and ecological half life of 347 days (Wolfe 1970a). To resolve this conflict it is necessary to distinguish between the long term average half life and the short term half life. If we assume that the loss of Zn from the oyster obeys first order reaction kinetics, then the loss rate is parameterized by the reaction constant, k. The magnitude of k will depend on the biological turnover of Zn and its diffusion across cellular membranes. Thus, k will not remain constant throughout the year and in fact will be a function of the metabolic rate of the oyster. Over short periods of time (several months) the biological half life of Zn (computed from k) can be significantly different from the long term average half life. The half life reported by Seymour is a long term average based on data which are not particularly sensitive to short time scale phenomena due to the low sampling frequency. Seymour's data have been reevaluated by Cutshall (1974) but by averaging uptake and loss curves from different seasons of the year short time scale, seasonal processes have still been lost. Wolfe also reported a long term average biological half life in the presence of some recycling of ^{65}Zn. However, Wolfe's data from 1965–1966 are detailed enough to resolve short term events. During August–September, 1965, the absolute ^{65}Zn activity decreased appreciably while the specific activity remained constant. An interpretation for these data could be a rapid bulk discharge of approximately 30% of the stable Zn body burden. In the following month, the ^{65}Zn absolute activity continued to decrease, but now with a concurrent decrease in specific activity. This implies that the bulk Zn flow had stopped, but the biological turnover of stable Zn continued at a high rate. Calculating a biological half life from Wolfe's data for the period July to October, 1965, gives roughly 45 days. Both the rate and magnitude of radioactive Zn loss during the summer months agree with the dynamics of stable Zn observed here. A consequence of this interpretation of Zn dynamics is that the biological half life of Zn is significantly increased during the winter months, and is in excess of one year.

Cu and Cd should be considered together with Zn because of the similarities of their seasonal cycles, their correlation with each other (Pringle *et al.* 1968; Windom and Smith 1972; Huggett *et al.* 1971), the relation between body burden-decrease in body burden-amount deposited in shell, and their lack of correlation with shell growth. Furthermore, Betzer and Pilson (1974) investigated the seasonal cycle of Cu in *Busycon canaliculatum*. They showed that although the concentration of Cu in the gonads was depressed during the summer as a result of increased gonadal weight, the body burden indeed follows a pattern similar to that exhibited by *Crassostrea virginica* and that the seasonal cycle of Cu body burden is highly correlated with gonadal index (fraction of total body weight). An interesting difference between Cd dynamics and Zn-Cu is the gradual increase in Cd throughout the winter months (water temperature less than 10 C) when little feeding occurs. This indicates that Cd is probably taken up directly from estuarine waters independently of food resources. The data of Kerfoot and Jacobs (1973) indicate that an ionic concentration in the range of 1–4 μg Cd/liter in the water would be sufficient to produce the observed increase in tissue concentration from September, 1971 to June, 1972. This water level is slightly elevated compared to reported concentrations of shoreline waters from the Irish Sea, 0.03–1.4 μg Cd/liter (Preston *et al.* 1972), but not so much as to be inconsistent with the conclusion that the oysters are accumulating Cd directly from the water.

The loss of Cd from oyster soft tissue has been reported to be very slow (Brooks and

Rumsby 1967; Kerfoot and Jacobs 1973). My data (Frazier, unpublished data) indicate that Cd may be bound 20 times stronger than Zn in oyster soft tissue. Thus, the long term, average biological half life of Cd could possibly be even greater than Zn.

The complexities of the metal dynamics in oyster soft tissues are too great to be explained by a single hypothesis. Most likely, all the hypotheses presented in the literature play some role in the ultimate control of the different metals in oysters. Since it is apparent that oysters at different locations have different metal concentrations (Coulson *et al.* 1932; Chipman *et al.* 1958; McFarren *et al.* 1961; Pringle *et al.* 1968; Kopfler and Mayer 1969; Windom and Smith 1972; Huggett *et al.* 1971), it can be suggested that the base line upon which the seasonal cycle is superimposed is set by local environmental conditions which determine the availability of metals in water and food resources while the seasonal cycle itself is controlled by biological activities. Whether feeding or spawning, shell metabolism or environmental physical chemistry is dominant at any given season will only be known when detailed experimental tests of the various hypotheses are performed.

Conclusions

The results of this research have shown that genetically similar populations of oysters can be employed as a valuable tool for studying details in the seasonal dynamics of metals which would otherwise have been masked by biological variation. Based on the observation of these hatchery reared oysters under natural conditions the following working hypotheses concerning the role of biological activities in metal dynamics are presented:

(1) Mn and Fe dynamics are closely related to shell growth processes. A high rate of turnover of Mn in soft tissue occurs during the shell growth season and tissue levels are controlled by the small difference between two high rates, the soft tissue uptake rate and the shell deposition rate.

(2) Zn, Cu, and Cd dynamics are closely related to gonadal development and spawning, either through direct incorporation into gametes or through functional requirements of gonadal tissue during development. The rapid discharge of these metals in the summer is due to biochemical and physiological readjustments related to gametogenesis.

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