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Chromium uptake and loss in the bivalves *Crassostrea virginica* and *Mytilus edulis**

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ABSTRACT: Chromium uptake and loss by the bivalves *Crassostrea virginica* and *Mytilus edulis* were studied at naturally occurring seawater temperature and salinity to determine their potential as an indicator of chromium pollution. After 12 wk treatment with 5 and 10 ppb Cr seawater, mean tissue concentration in oysters, *C. virginica*, was 3.12 ± .45 and 5.63 ± 1.15 μg Cr g^{-1} dry weight, respectively. Mean chromium concentration in mussels, *M. edulis*, treated with 5 and 10 ppb Cr seawater for 12 wk were 4.83 ± 1.32 and 9.41 ± 3.37 μg Cr g^{-1} dry weight, respectively. Significant linear relationships exist between chromium uptake by oysters and mussels and seawater chromium concentrations over the range of concentrations used in this study. A significant inverse relationship exists between tissue chromium concentration and dry weight in both oysters and mussels. Chromium concentration in oysters continued to increase during spawning, whereas it decreased in mussels. After holding chromium treated mussels in ambient flowing seawater for 28 wk, a 61 and 70% loss of chromium occurred in mussels treated with 5 and 10 ppb Cr, respectively. When treated similarly, oysters from both the 5 and 10 ppb Cr treatments lost 42% of their tissue chromium after 28 wk depuration. Evidence is presented which suggests that oysters would be a better indicator of chromium pollution of the environment than mussels.

INTRODUCTION

Chromium has long been an item of commerce and has great economic importance. Despite its long history, many aspects of the element, particularly its interaction with the environment, remain obscure.

Compared to trivalent chromium compounds, hexavalent chromium compounds have the greater economic importance as well as the greater biological and environmental significance (Towill et al., 1978). Sodium dichromate is the leading commercial form of chromium and almost all other chromium compounds are prepared from it. Therefore, this chromium compound more than any other is probably more likely to enter estuaries.

Ambient chromium concentrations in seawater are low, with a range of 0.25 to 0.5 μg l^{-1} (Bond et al., 1973). Hexavalent chromium rarely occurs naturally because it is readily reduced in the presence of organic matter. However, after introduction by man, hexavalent chromium often remains unchanged in many natural waters because of low concentrations of reducing matter (Mertz et al., 1974). Chromium in seawater can be either tri- or hexavalent; however, Fukai (1967) suggested that the stable chromium species in seawater was hexavalent.

Chromium is released into the environment from many sources, and small amounts are taken up by a wide variety of organisms (Towill et al., 1978). Although environmental interactions of chromium have not been studied adequately, chromium does not appear to be biomagnified in aquatic food chains despite its accumulation by certain organisms (Baptist and Lewis, 1969; Mathis and Cummings, 1973). Chromium uptake by oysters has been reported by Pringle et al. (1968) and Shuster and Pringle (1969). However, they treated oysters with trivalent chromium under laboratory conditions and with chromium concentrations (0.05 to 0.1 mg l^{-1}) that far exceeded those expected in the environment.

Since little is known about the impact of low chromium concentrations on oysters and mussels, we initiated studies in which *Crassostrea virginica* and *Mytilus edulis* were treated with 2 concentrations of

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hexavalent chromium under otherwise ambient seawater conditions. Our study focused on the kinetics of bioaccumulation and depuration of hexavalent chromium by oysters and mussels over an extended period of time and to determine if either species would be a good biological indicator of chromium in nearshore marine waters.

MATERIALS AND METHODS

Test organisms were adult oysters *Crassostrea virginica* Gmelin obtained from Cotuit Sound, Massachusetts, USA, and adult mussels *Mytilus edulis* Linnaeus obtained from Narragansett Bay, Rhode Island, USA, in May 1979. The mean height of the oysters was 9.5 ± 0.3 cm and that of the mussels was 6.7 ± 1.5 cm. Histopathologic examination, which was used to determine the general health condition of oysters and mussels at the time of harvest and at the start of chromium addition to the experimental troughs, did not show any abnormalities. Oysters and mussels were acclimatized for 1 mo in fiberglass troughs with a flow-through seawater system using unfiltered Narragansett Bay water. The troughs measured 3.75 m long × 30 cm wide × 25 cm deep and were supplied with flowing unfiltered seawater (29 to 32 ‰ S) at the rate of 20 l min^{-1}. Each trough held either 50 oysters or 50 mussels on a false bottom consisting of polyethylene grids resting 2.5 cm off the trough bottom on PVC pipe. The oysters and mussels did not receive supplementary feeding during the holding or treatment periods. The ambient seawater temperature was continuously recorded during the experiment.

Six troughs holding oysters (3 per chromium treatment) received a sodium dichromate solution ($Na_2Cr_2O_7 \cdot 2H_2O$) and 3 troughs served as controls. A duplicate system for mussels was used. A deionized water stock-solution was pumped into the troughs at a rate, which when mixed with incoming seawater (20 l min^{-1}), produced a calculated concentration of 5.0 or 10.0 ppb Cr.

Five oysters and mussels were removed from each treatment and the tissue chromium concentration determined prior to dosing with chromium in June (Time 0). During the accumulation period (June to August), 5 oysters and 5 mussels were removed from each treatment biweekly for 12 wk and analyzed for chromium. The first sample in the depuration study was taken after 14 wk (2 wk after termination of dosing) and biweekly thereafter to Week 20.

Chromium concentrations in the experimental troughs were determined by heated graphite atomization (HGA) using a Perkin-Elmer Model HGA 2100 coupled with a Perkin-Elmer Model 360 atomic absorption (AA) spectrophotometer. Prior to analysis, 100 µl of concentrated HNO_3 (ultra-pure grade) were added to 1 ml of each of the test samples. Standards for the chromium determinations were prepared in ambient seawater to match salinities of the test solutions in order to provide matrix matching and eliminate differences between standards and test samples. Replicate seawater samples were determined for each chromium concentration. Each sample and standard were injected twice into the HGA unit and the average concentration with its standard deviation was determined. The detection limit for chromium with this technique is approximately 1 µg kg^{-1} of seawater. Seawater in the control troughs contained a mean concentration less than 1 ppb (i.e. detection limit). The mean concentrations in the troughs holding oysters were 5.13 ± .43 (range 4.20 to 5.81) and 10.60 ± .66 (range 8.83 to 11.62) for the 5 and 10 ppb Cr treatments, respectively. Those troughs holding mussels had mean concentrations of 5.05 ± .35 (range 4.06 to 5.91) and 10.06 ± .53 (range 8.70 to 11.61) for the 5 and 10 ppb Cr treatments, respectively. No chemical separation or matrix modification techniques were used to enhance the detection limit for chromium.

The total soft parts of oysters and mussels to be analyzed by atomic absorption spectrophotometry were removed from the shells and oven-dried at 100 °C prior to wet ashing in concentrated HNO_3 (reagent grade). Tissue samples were digested in concentrated HNO_3 at 80 °C for several days. All soluble organic compounds were decomposed with careful addition of 30 % H_2O_2 to the HNO_3 solutions after the initial digestion period. The clear solution obtained with repeated addition of H_2O_2 was concentrated to approximately 2 ml and reconstituted with 5 % HNO_3. The solution was filtered through Whatman #42 filters (previously washed with 5 % HNO_3) and brought to final volume (50 ml) with 5 % HNO_3. Blanks were processed concurrently with each set of 15 tissue samples. The blank samples were subjected to the same rigorous treatment as the tissue samples (i.e. HNO_3, H_2O_2 and filtration).

Calibration of the AA spectrophotometer was performed before and after the analysis of 30 samples and recalibration checks were performed after every 5 samples. Each sample and standard were injected at least 3 times into the HGA unit to determine the reproducibility of the measured signal and the mean of the 3 determinations was used to compute the concentrations for each sample. A minimum of 10 % of all samples was determined by standard addition. Matrix effects were found to be non-existent in the tissue samples since differences greater than 10 % were not obtained for any of the samples analyzed by the standard comparison method. Blank samples were generally undetectable and contributed less than 10 % to the lowest value reported.

Regression analyses were performed to determine the influence of seawater chromium concentration, time and weight on uptake and depuration of chromium by oysters and mussels.

RESULTS

The mean concentration of chromium in oysters and mussels was significantly ($P < 0.05$) different between treatments. As much as $3.12 \pm .45$ and 5.63 ± 1.15 µg Cr g^{-1} dry weight were accumulated by adult oysters from seawater containing 5 and 10 ppb Cr, respectively, after 12 wk treatment (Fig. 1). Adult mussels

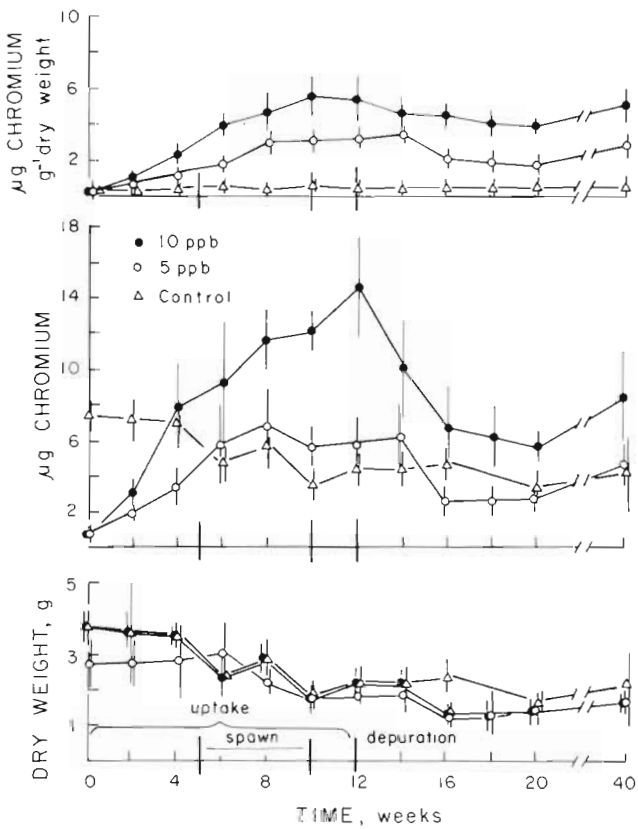

Fig. 1. *Crassostrea virginica*. Dry weight, chromium content and chromium concentration in the total soft parts of oysters treated with ambient (control) 5 and 10 ppb chromium seawater for 12 wk (June to August, 1979). Each symbol represents the mean value from each sample of 5 oysters ± 1 standard deviation

contained 4.83 ± 1.32 and 9.41 ± 3.37 µg Cr g^{-1} dry weight after 12 wk treatment with seawater containing 5 and 10 ppb Cr, respectively (Fig. 2).

Linear regression analyses showed that slopes for time of treatment increased significantly ($P < 0.05$) for oysters but not for mussels as the chromium concentration increased in the troughs. In the 5 ppb Cr treatment, tissue chromium concentration increased significantly ($P < 0.01$) at the same rate in oysters and mussels. However, the rate of increase of tissue chromium concentration in mussels treated with 10 ppb Cr was not significantly ($P > 0.05$) different from that obtained with the 5 ppb Cr treatment. In contrast, the rate of tissue concentration increase in oysters ($P < 0.01$) treated with 10 ppb Cr seawater was almost double that obtained with the 5 ppb Cr treatment.

The coefficient of determination (R^2) for the significant ($P < 0.05$) linear models of chromium uptake by the oysters is 0.87 for both the 5 and 10 ppb Cr treatments. In contrast, the R^2 values for the significant ($P < 0.05$) linear models of chromium uptake by mussels are 0.71 and 0.50 for the 5 and 10 ppb Cr treatments, respectively. Therefore, it appears that chromium uptake by the oyster is better fit by a linear model than that of the mussel.

Uptake patterns expressed as chromium concentration (µg g^{-1}) suggested that chromium increased continuously in oyster tissues over the 12 wk uptake period (Fig. 1). Oysters continued to accumulate chromium during their natural spawning period which was observed in the experimental troughs and confirmed by histopathological examination during Weeks 5 to 10 (Fig. 1). Uptake, expressed as content (µg) decreased during Week 10 which coincided with spawning in the 5 ppb Cr treatment. However, uptake as content continued to increase in the oysters through the spawning period in the 10 ppb Cr treatment (Fig. 1).

In contrast, chromium uptake, as concentration in mussels, decreased during their natural spawning period, which was observed in the experimental troughs and confirmed by histopathological examination during Weeks 5 to 10 (Fig. 2). Although a decrease in chromium content and dry weight did occur during spawning in the control mussels and those treated with 5 ppb Cr, these decreases were not as apparent as those observed in the 10 ppb Cr treatment (Fig. 2).

A significant ($P < 0.05$) inverse relationship was observed between chromium concentration in the soft tissues and dry weight; whereas, a significant ($P < 0.05$) direct relationship existed between chromium content and dry weight in both oysters and mussels. Linear regression analyses indicated that in the control, 5 and 10 ppb Cr treatments there was no significant ($P > 0.05$) change in dry weight of mussels during the uptake period (Weeks 0 to 12; Fig. 2). In addition, no significant ($P > 0.05$) change in dry weight of mussels from all treatments occurred during the spawning period (Weeks 5 to 10). Although it appeared that oysters were losing weight during the uptake period, linear regression analyses indicated that no significant ($P > 0.05$) change in dry weight of oysters

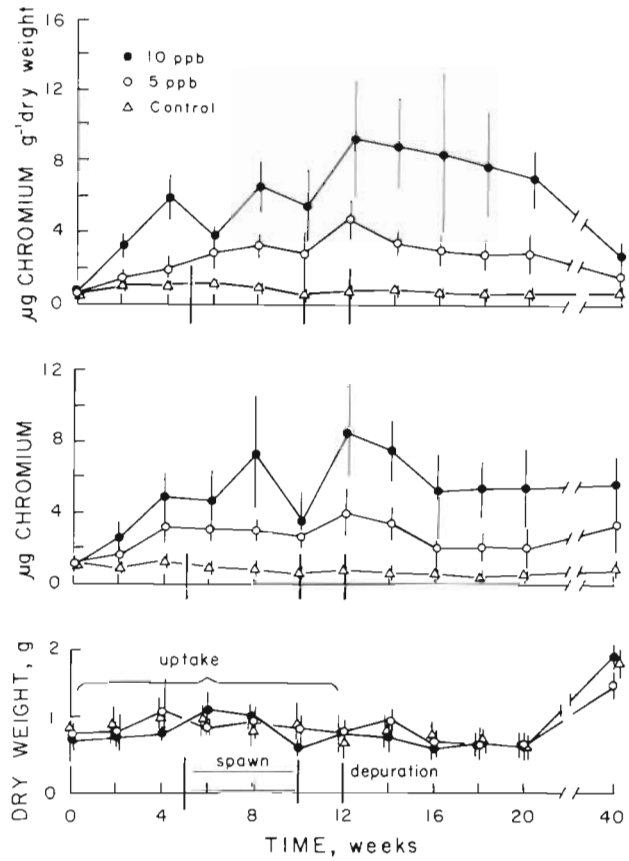

Fig. 2. *Mytilus edulis*. Dry weight, chromium content and chromium concentration in the total soft parts of oysters treated with ambient (control), 5 and 10 ppb chromium seawater for 12 wk (June to August, 1979). Each symbol represents the mean value from each sample of 5 oysters ± 1 standard deviation

from the control and 5 ppb Cr treatments occurred during chromium uptake (Weeks 0 to 12) (Fig. 1). However, linear regression analyses indicated that a significant ($P < 0.01$, $R^2 = 0.56$) loss in dry wt occurred during the uptake period (Weeks 0 to 12) in oysters treated with 10 ppb Cr (Fig. 1). In addition, linear regression analyses suggested that dry weight loss in oysters during spawning (Weeks 5 to 10) was significant ($P < 0.05$) in the control, 5 and 10 ppb Cr treatments.

Chromium concentration and content in mussels treated with 10 ppb Cr continued to decrease throughout the 8 wk depuration period (Weeks 12 to 20; Fig. 2). Chromium concentration in mussels treated with 5 ppb Cr did not continue to decrease after 6 wk depuration (Week 18; Fig. 2). After treatment with 5 ppb Cr for 12 wk, mussels lost 28 % of their tissue chromium concentration within the first 2 wk after chromium addition to the troughs was discontinued (Fig. 2). An additional 21 % chromium was lost during the following 4 wk depuration (Weeks 14 to 18) for a total chromium loss of 49 % over the 6 wk period (Fig. 2). In mussels treated with 10 ppb Cr, a 9 % loss in chromium concentration was observed 4 wk after chromium addition to the troughs was terminated; whereas, during the next 4 wk, 25 % of the chromium was lost to give a total chromium loss of 34 % over the 8 wk depuration period (Fig. 2).

In an attempt to determine the maximum loss of chromium from mussels, those mussels which remained after 20 wk of study were allowed to depurate in ambient flowing seawater (20 l min^{-1}) for an additional 20 wk. Thus, 28 wk after chromium addition to the troughs was terminated, a 61 and 70 % loss of chromium occurred in mussels treated with 5 and 10 ppb Cr, respectively.

Although body burdens were higher in mussels treated with 10 ppb Cr than in those treated with 5 ppb Cr, analyses of covariance (Snedecor and Cochran, 1967) which were used to test slope equality indicated that when chromium loss occurred (Weeks 12 to 20) the rates of loss were not significantly ($P > 0.05$) different between treatments. Although the rate of chromium loss in mussels is the same between treatments, the amount of chromium loss differs significantly ($P < 0.05$). Thus the same percentage chromium loss occurs in mussels from both treatments but the body burdens remain higher in the mussels treated with 10 ppb Cr than in those treated with 5 ppb Cr.

Tests for slope equality (analyses of covariance) indicated that significant differences do not exist ($P > 0.05$) between rate of chromium loss as concentration and content in mussels treated with 5 ppb Cr. These same analyses suggested that in mussels treated with 10 ppb Cr, the rate of chromium loss as content was significantly ($P < 0.05$) greater than chromium loss as concentration (slopes of regression lines for chromium loss as content and concentration are −0.36 and −0.28, respectively). Weight fluctuations do not appear to be responsible for the differences in rates of chromium loss since weight remained stable during the depuration period (Weeks 12 to 20; Fig. 2).

During the first 4 wk of depuration when seawater temperatures were in excess of 20 °C, the chromium concentration decreased by 43 and 9 % in mussels treated with 5 and 10 ppb Cr, respectively (Figs. 2 and 3). During the following 24 wk, seawater temperatures decreased continuously, and tissue chromium concentration decreased an additional 18 and 41 % in mussels treated with 5 and 10 ppb Cr, respectively (Figs. 2 and 3). Chromium concentration in oysters treated with 5 ppb Cr did not decrease during the first 2 wk of depuration (14 wk); however, after 4 wk depuration, chromium concentration decreased by 36 % (Fig. 1). Oysters treated with 10 ppb Cr, unlike those treated with 5 ppb Cr, lost chromium during the first 2 wk

depuration and lost 18 % chromium after 4 wk depuration (Fig. 1). A 50 % loss of chromium from oysters treated with 5 or 10 ppb Cr was not observed up to 8 wk depuration.

In an attempt to determine the maximum loss of chromium from oysters, those oysters which remained after 20 wk of study were allowed to depurate in ambient flowing seawater (20 l min^{-1}) for an additional 20 wk. After 28 wk depuration, a 42 % decrease in chromium concentration occurred in oysters from both the 5 and 10 ppb Cr treatments.

Although chromium body burdens were higher in oysters treated with 10 ppb Cr than in those treated with 5 ppb Cr, tests for slope equality (analyses of covariance) indicated that the rates of chromium loss were not significantly different ($P > 0.05$) between treatments. Although the rate of chromium loss in oysters is the same between treatments, analyses of covariance suggested that the amount of chromium lost differs significantly ($P < 0.05$) between treatments. Thus, the same percentage chromium loss occurs in oysters from both treatments, but body burdens remain higher in oysters treated with 10 ppb Cr than in those treated with 5 ppb Cr after 28 wk depuration. The rate of chromium loss as content is significantly greater ($P < 0.05$) than chromium loss as concentration in oysters from both the 5 and 10 ppb Cr treatments.

During the first 4 wk of depuration (Weeks 12 to 16) when seawater temperatures were in excess of 20 °C, the chromium concentration in oyster tissue decreased 38 and 18 % in the 5 and 10 ppb Cr treatments, respectively (Figs. 1 and 3). Seawater temperature decreased continuously for the following 24 wk depuration; and at the end of this period, chromium concentration in oysters treated with 5 and 10 ppb Cr decreased an additional 6 and 24 %, respectively.

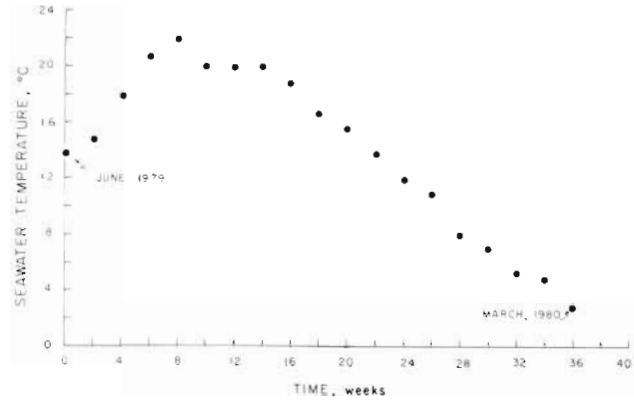

Fig. 3 *Crassostrea virginica*. Weekly mean seawater temperature during chromium treatment and depuration

DISCUSSION

Though our results indicate that chromium uptake in *Crassostrea virginica* and *Mytilus edulis* is linear, Shuster and Pringle (1969) reported that chromium accumulation by *C. virginica* was curvilinear and occurred in 3 phases when temperature was maintained at 20 °C for 20 wk. The logarithmic plots of our oyster and mussel uptake data were similar to that reported by Shuster and Pringle (1969); however, 3 phases of uptake were not apparent in our plots.

Ayling (1974) suggested that oysters absorb chromium by a physiological process up to a maximum concentration that depended upon the size of the oyster. In addition, Ayling (1974) reported that chromium concentration in *Crassostrea gigas* is not related to size; whereas, the total content is directly related to size. In this study, tissue chromium concentration is inversely related with weight and tissue chromium content directly related with weight. This is not unique to this study since similar findings have been reported (Cunningham and Tripp, 1975; MacKay et al., 1975; Watling and Watling, 1976; Zaroogian, 1980). Fluctuations in metal concentrations in *Mytilus edulis* have been attributed to variations in wet weight by Phillips (1976). It appeared that mussels with high wet weights had low concentrations of trace metals. It was suggested that total metal content remained almost constant throughout the year but that the mussels' weight change caused fluctuations in metal concentrations in the soft tissues. Although we observed higher chromium contents in larger than in smaller mussels, a significant relationship did not exist between concentration and dry weight.

Ayling (1974) stated that tissue concentrations of chromium in oysters is almost independent of the metal concentration at each site. In this study, oysters continued to accumulate chromium for up to 12 wk with no indication that accumulation would not proceed with the continued addition of chromium to the seawater. We found that tissue chromium concentration is dependent upon seawater chromium concentration in both oysters and mussels. Shuster and Pringle (1968) also reported a similar relationship between seawater concentration and tissue concentration for chromium.

Shuster and Pringle (1969) used chromium concentrations (50 and 100 µg l^{-1}) much higher than those employed in this study. Differences in uptake curves could be explained by the fact that Shuster and Pringle (1969) used trivalent chromium which is essentially insoluble in seawater and would be present as a particulate. Hexavalent chromium, which is soluble in seawater, was used in this study.

The logarithmic plots of the mussel data were not as clearly defined as the oyster plots. Uptake of chromium

by mussels during this study was not continuous, particularly during the last 4 wk of uptake. This could account for the poor fit of the logarithmic plots. Linear regression analyses of Shuster and Pringle's (1969) data indicated that chromium accumulation by *Crassostrea virginica* is a significant ($P < 0.01$) linear function of time. In addition, the rate of chromium uptake in their oysters treated with 50 $\mu g\ l^{-1}$ was extremely close to the rate obtained in this study when oysters were treated with 5 ppb Cr (slope coeff. 0.29 and 0.28, respectively).

Less variability was observed with tissue chromium concentration among oysters than among mussels in samples collected during the accumulation period. The spawning process could contribute to the variability, since losses both in chromium content (μg) and concentration ($\mu g\ g^{-1}$) were observed in mussels and not oysters. Losses in chromium concentration were also expected in oysters during spawning since it has been reported that metal uptake is interrupted in oysters during spawning (Cunningham and Tripp, 1973; Zaroogian et al., 1979; Zaroogian, 1980).

A greater loss in weight than in chromium content could be responsible for the continuous increase in chromium concentration observed during spawning. Weight losses (16 %) in the 5 ppb Cr treated oysters were greater than the losses in content (13 %) which would account for the increase in concentration during spawning. During this same period, the percentage increase in content (21 %) in the 10 ppb Cr treated oysters was more than twice the increase in weight (10 %) which could also account for an increase in concentration.

The continual increase in chromium content observed only in oysters treated with 10 ppb Cr during the spawning period could be due to the greater diffusion and accumulation of chromium interstitially at higher seawater chromium concentrations, thus resulting in increased chromium body burdens. If we assume that this unbound or loosely bound chromium would be lost from the tissues more rapidly than bound chromium upon termination of chromium addition, then the more rapid loss of chromium from oysters treated wih 10 ppb Cr than with 5 ppb Cr observed during the first 2 wk of depuration could be explained. As much as 32 % chromium accumulated by oysters treated with 10 ppb Cr was lost after 2 wk depuration whereas no chromium loss from oysters was apparent during this period in those treated with 5 ppb Cr. Similarly, 29 % of the chromium in mussels treated with 10 ppb Cr was lost after 2 wk depuration compared to a 0.1 % loss in 5 ppb Cr treated mussels.

Pringle et al. (1968) suggested that the initial tissue concentration of a given metal appears to be directly related to the depletion rate for a given species. Schulz-Baldes (1974) reported that the rate of lead loss from *Mytilus edulis* is closely correlated with the internal lead concentration. In the case of mercury loss from *Crassostrea virginica*, Cunningham and Tripp (1975) established that in a declining temperature regime greater losses occurred in oysters that received higher concentrations of mercury. Under conditions of a natural temperature decline, more than 54 % of the lead concentration in *C. virginica* was lost after 4 wk and loss did not appear to be constant (Zaroogian et al., 1979). Our findings indicated that the rate of chromium loss as concentration in oysters and mussels is not related to tissue concentration and loss appeared to occur at a constant rate.

In addition, it appears that chromium depuration is not entirely time and temperature dependent. Rather, chromium loss occurs slowly in oysters and mussels until a residual concentration of chromium in the tissue is attained, after which no chromium loss appears to occur.

The biological half-life of a metal is defined as the time required for half the concentration of accumulated trace metal to be lost (Renfro, 1973). Using the equation of Renfro (1973) for calculating biological half-life, times of 2 and 1 wk were obtained for oysters and mussels, respectively. However, during 28 wk depuration, a 50 % loss in tissue chromium concentration was not observed in either species.

Butler et al. (1971) and Phillips (1976) recommended certain requisites for a biological indicator of metals. We feel that both *Crassostrea virginica* and *Mytilus edulis* satisfy the requirements for chromium. However, mussels appear to be better accumulators of chromium with their higher tissue concentrations in both treatments. Although the oysters accumulate less chromium than mussels, the individual variability is much less than in mussels. In addition, it appears that spawning does not affect uptake in oysters as it does in mussels. On these bases, it would appear that *C. virginica* would be a better indicator of chromium in its environment.

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