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Field Studies Using the Oyster *Crassostrea virginica* To Determine Mercury Accumulation and Depuration Rates

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Mercury as an environmental hazard, especially with regard to human health, has been of concern since the Minamata disaster (Huddle et al. 1975). From 1966 to 1970 a chlor-alkali plant in Point Comfort, Texas released mercury-enriched wastewater (up to 29.9 kgHg/day) into Lavaca Bay (TWQB 1977). Since 1970 the Texas Department of Health (TDH) has periodically closed and then re-opened portions of Lavaca Bay to the harvesting of crabs and finfish based on their levels (< >0.5 ppm Hg wet weight) of mercury. A 1988 closure remains in effect as of this writing (Wiles, 1993). Mercury contamination in Lavaca Bay organisms thus continues to be a problem 22 years after the chlor-alkali plant ceased releasing mercury into the bay. The goal of the following research was to better understand the behavior of mercury in Lavaca Bay.

Oysters have been widely used as indicator species in metal pollution studies (Goldberg et al. 1983). Most such programs have focused on the concentrations of metals in oysters from different geographic areas. This study, however, investigated the rate and amount of mercury a "clean" oyster would accumulate when transplanted to a contaminated estuary and the rate of mercury depuration by contaminated oysters placed in a clean environment. The oysters were additionally analyzed for Ba, Cu, Fe, P, and Zn to test for the possible involvement of these metals in mercury accumulation and depuration.

MATERIALS AND METHODS

In August 1991, mature Crassostrea virginica were collected from an uncontaminated area, Carancahua Reef, in Carancahua Bay, Texas for use in the accumulation study. The oysters were placed in nylon bags with a mesh size of 0.5 cm. Each bag contained about 50 oysters. The bags were taken about 16 km away to Lavaca Bay and a control site, Keller Bay (see Fig. 1) and placed on plastic grates, which prevented them from sinking into the soft sediment at the 1 m deep sites. Nine transplanted oysters from each site were collected on days 0, 7, 14, 21, 36, and 51, placed in plastic bags, and frozen for later analysis.

The depuration study, also conducted in August 1991, used C. virginica from

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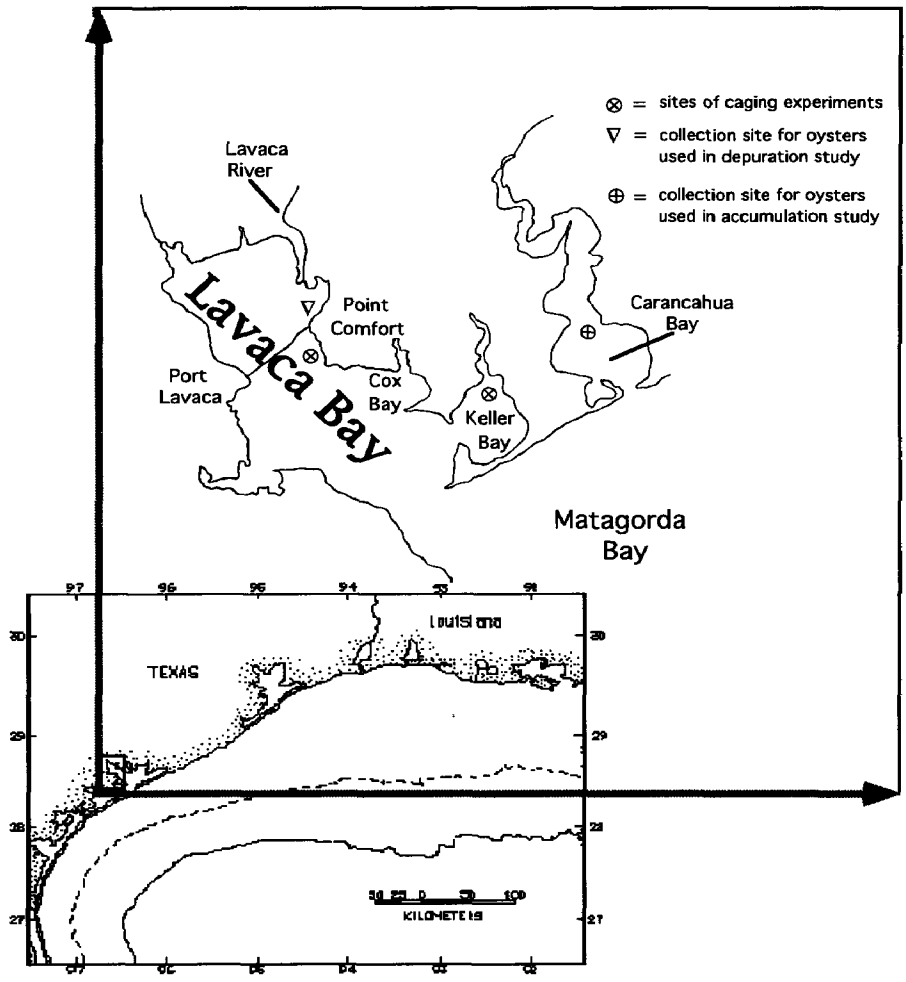

Figure 1. Map of Lavaca Bay showing caging sites and oyster collection locations for the accumulation and depuration experiments.

an area of North Lavaca Bay known to be contaminated with Hg. These oysters were placed in a contaminated area of lower Lavaca Bay, as a control site, and in uncontaminated Keller Bay (see Fig. 1). Deployment techniques and collection times were the same as those used in the accumulation study.

On each sampling date oysters were removed from the experimental bags in both the accumulation and depuration experiments and nine individuals from the natural population of C. virginica at the respective sites were collected.

In the lab, oysters were thawed and opened with a stainless steel knife. The soft tissue was removed with a teflon spatula and plastic forceps, rinsed in

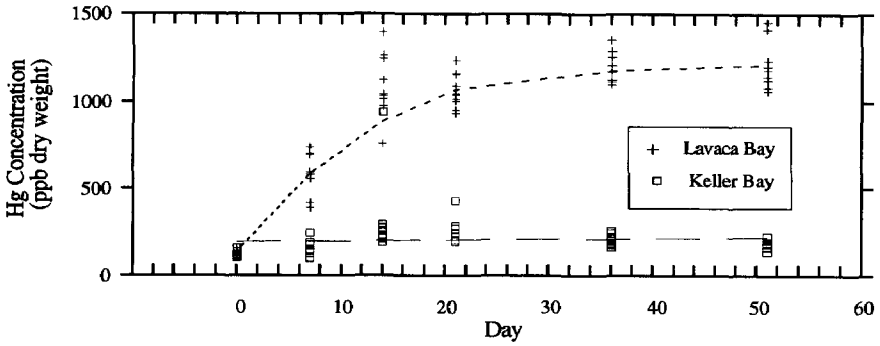

Figure 2. Mercury concentrations in Carancahua Reef oysters transplanted to Lavaca and Keller Bays for the 51-d accumulation experiment.

Table 1. Certified and experimental data for National Bureau of Standards reference material 1566a Oyster Tissue and the elemental detection limits obtained in this study.

Element	Hg (ppb)	Ba (ppm)	Cu (ppm)	Zn (ppm)	P (ppm)	Fe (ppm)
NBS Certified 1566a Oyster Tissue	64.2 ± 6.7	NC	66.3 ± 4.3	830 ± 57	6230 ± 180	539 ± 15
Experimental 1566a Oyster Tissue (n=15)	55.7 ± 2.0	1.49 ± 4	73.8 ± 4.7	935 ± 36	6203 ± 260	549 ± 43
Detection Limit	6	0.4	10	90	680	110

NC = not certified

distilled-deionized water, weighed, freeze dried, and homogenized before analysis. All oysters were individually digested according to a modification of the USEPA 245.1 (USEPA 1990) method and analyzed for mercury by cold vapor atomic absorption spectrophotometry (Hatch and Ott 1968). Samples were additionally analyzed for Ba, Cu, Fe, P, and Zn using a modified Applied Research Laboratories, Inc., (ARL) SpectraSpan® VI Direct Current Argon Plasma (DCP) Emission Spectrophotometer following ARL's instructions (ARL 1991). Every digest (about 30 samples) included two aliquots of the reference material, 1566a Oyster Tissue, certified by the National Bureau of Standards. The certified and experimental values from the 1566a Oyster Tissue and the detection limits for each element are listed in Table 1.

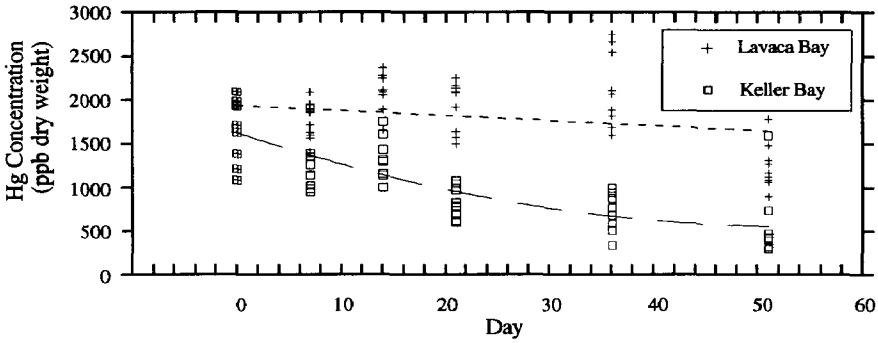

Figure 3. Mercury concentrations in oysters collected from North Lavaca Bay when transplanted to lower Lavaca and Keller Bays for the 51 day depuration experiment.

Statistical analyses using SAS Institute, Inc. software (SAS INSTITUTE INC. 1985) were performed on the data from the three oyster groups, accumulation (AA), depuration (DD), and the natural population (NP) to investigate possible relationships among the variables analyzed. The Spearman correlation test and the general linear model (GLM) were used to find correlations and linear relationships among variables. The dry weight of the oysters was used as a covariant in the GLM. The Least Square Means test, LSMEANS, was used to verify changes in Hg with time during the caging experiments.

RESULTS AND DISCUSSION

Oysters removed from Carancahua Bay readily accumulated mercury when placed in Lavaca Bay. Mercury accumulation was rapid through the first 14 days of exposure and leveled off with time (Fig. 2). The rate of Hg accumulation between days 0 and 14 averaged 70 ppb Hg per day. From day 15 to 51 mean daily Hg uptake ranged from 0 to 10 ppb. The Hg levels in the control oysters from Carancahua Bay did not significantly change over the 51-d experiment when caged in Keller Bay. The GLM procedure showed a significant (p <0.05) difference in Hg levels between sites and days collected.

The LSMEANS test showed that Hg concentrations in oysters caged in Lavaca Bay on days 7, 14, 21, 36, and 51 were all significantly higher than day 0 at p <0.05, while the Hg levels on days 14, 21, 36, and 51 were not significantly different from one another.

The results of the depuration study showed that contaminated oysters released Hg when placed in an uncontaminated environment, Keller Bay (Fig. 3). The average Hg level dropped from 1660 ± 363 ppb on day 0 to 550 ± 416 ppb on day 51. The rate of Hg depuration varied throughout the experiment. The rate was highest on days 14 to 21, averaging a loss of 72 ppb Hg per day. According to the LSMEANS test, concentrations in oysters caged in Keller Bay on days 21, 36, and 51 were significantly lower than Hg levels in oysters from North Lavaca Bay transplanted to Keller Bay on day 0 at p <0.05.

Table 2. Correlations among variables measured in the oyster accumulation and depuration experiments and the natural populations in Keller (KB) and Lavaca Bay (LB). Upper, Spearmans rho; lower, P value

Variables	Accumulation		Natural Population		Depuration	
	KB	LB	KB	LB	KB	LB
Cu and Zn	0.83100 (<0.0001)	0.81849 (<0.0001)	0.91104 (<0.0001)	0.87307 (<0.0001)	0.89350 (<0.0001)	0.82946 (<0.0001)
Zn and P	0.40244 (0.003)	0.42855 (0.001)	0.28371 (0.04)	0.40736 (0.002)	0.32464 (0.02)	0.37663 (0.005)
Ba and Fe	0.64172 (<0.0001)	0.62662 (<0.0001)	0.60290 (<0.0001)	0.68725 (<0.0001)	0.51649 (<0.0001)	0.49675 (<0.0001)
Cu and P	0.37744 (0.005)	0.41719 (0.002)	NS	0.36245 (0.002)	0.36627 (0.007)	0.49388 (<0.0001)
P and Fe	0.35252 (0.01)	NS	0.28380 (0.04)	NS	0.41407 (0.002)	0.33303 (0.01)
Dry Wt. and Hg	NS	-0.40646 (0.002)	-0.48637 (0.0003)	NS	-0.46336 (0.0004)	-0.38954 (0.004)
Hg and Cu	NS	NS	NS	0.55418 (<0.0001)	0.66808 (<0.0001)	0.29423 (<0.0001)
Hg and Zn	NS	NS	NS	0.56905 (<0.0001)	0.70215 (<0.0001)	0.43221 (<0.0001)
Cu and Fe	NS	NS	NS	0.26899 (0.05)	0.49198 (0.0002)	0.37663 (0.005)

NS = Not significant

The average oyster living in the transplant sites in Lavaca and Keller Bays contained 2068 ± 676 ppb Hg and 354 ± 124 ppb Hg, respectively. The Carancahua Bay oysters caged in Lavaca Bay for the accumulation experiment increased dramatically in Hg concentration but did not reach the high average Hg levels in the natural population of oysters in Lavaca Bay. Similarly, oysters caged in Keller Bay for the depuration experiment decreased in Hg from 1660 ± 363 to 550 ± 416 but did not acquire the low Hg concentrations found in the natural population of oysters in Keller Bay.

The transplant experiments clearly show that C. virginica rapidly accumulated mercury when placed in a contaminated environment, Lavaca Bay. Furthermore, mercury-contaminated C. virginica from Lavaca Bay were found to quickly depurate Hg when placed in an uncontaminated environment, Keller Bay. Although the initial rate of Hg uptake was much faster than was the release, the oysters in the accumulation and depuration experiments both changed average Hg levels by about 1000 ppb over the 51-d experiment. The results of this study confirm earlier work (Riegal 1990)

showing that Hg contamination is a continuing problem in Lavaca Bay and that the Hg is readily available for bioaccumulation by oysters.

Positive correlations between Cu and Zn, Zn and P, and Ba and Fe were found in every experiment (Table 2). Copper and phosphorus were positively correlated in every oyster group except the natural population from Keller Bay. A positive relationship between P and Fe and a negative correlation between Hg and oyster dry weight were found in several experiments. Correlations were also seen between Hg and Cu, Hg and Zn, and Cu and Zn in oysters from Lavaca Bay, i.e., those collected for the depuration experiment and the natural population of oysters from Lavaca Bay.

Positive relationships between Cu and Zn in bivalves such as those found here have been noted in previous studies (Páez-Osuna and Marmolejo-Rivas 1990a, Marcus and Thompson 1986, Wright et al. 1985, and Páez-Osuna and Marmolejo-Rivas 1990b). Copper and zinc are both biologically active elements, but the reason for their strong correlation is not understood. Previous work (George and Pirie 1980) found that Zn transferred in the plasma of Mytilus edulis was mostly associated with granules that also contained Fe, S, P, K, and Ca. Others have indicated that M. edulis sequesters Zn and Fe in lysosomes in various cell types (Lowe and Moore 1979). George et al. (1978) found Zn and Cu were immobilized in individual granular amoebocytes; granular cells which contained Cu and Zn were present in Ostrea edulis, O. angasi, and C. gigas. It is believed that oysters concentrate Cu, Zn, P and other metals in granules to detoxify and change them to an excretable form (George and Pirie 1980, George et al. 1978). The correlations between Cu and Zn, Cu and P, and Zn and P found in this study could be related to granular formation in C. virginica, but no documentation of this was obtained.

The relationship among the elements Hg, Zn, and Cu may be a result of the high Hg concentrations in the water and sediment in Lavaca Bay. Perhaps Cu and Zn function in protective mechanisms in the detoxification of Hg in C. virginica. Another possible explanation is that Lavaca Bay oysters contain more metal-binding granules or low molecular weight binding proteins, metallothioneins, which could detoxify the metals (Lobel and Payne 1984). The strongest correlation between the three elements was found in oysters in the depuration study, where Cu and Zn closely followed the trend of decreasing Hg with time.

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