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Effects of Whole Sediments from Corpus Christi Bay on Survival, Growth, and Reproduction of the Mysid, *Americamysis bahia* (Formerly *Mysidopsis bahia)*

G. M. Cripe,[1] R. S. Carr,[2] S. S. Foss,[1] P. S. Harris,[1] R. S. Stanley[1]

[1] U.S. Environmental Protection Agency, Gulf Ecology Division, 1 Sabine Island Drive, Gulf Breeze, FL 32561, USA
[2] U.S. Geological Survey, Biological Resources Division, MERS, TAMU-CC, Center for Coastal Studies, Natural Resources Center, Suite 320, 6300 Ocean Drive, Corpus Christi, TX 78412, USA

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Estuarine and marine coastlines are receiving waters containing many anthropogenic substances. Concentrations of many of these contaminants have been diminished by regulatory control of effluents, but there is concern that continuing inputs (non-point sources) and contaminants contained in submerged sediments may have substantial impacts on the aquatic benthic communities that use them (Gess and Pavlostathis 1997).

Assessment of the biological impacts of contaminated sediments is critical to evaluate the magnitude of our coastal problems of in-place sediments, as well as to manage risk from those sediments proposed for disposal from dredging operations. A variety of infaunal organisms, including several amphipod species, have been used to evaluate sediment impacts on benthic communities (Breteler et al. 1989, Carr et al. 1996, 1998, DeWitt et al. 1992, Swartz et al. 1997). These organisms are usually exposed to the sediments for a duration of 10 days under static conditions, with survival as the endpoint.

One of the estuarine species most frequently employed in contaminant assessments is the mysid, *Americamysis bahia.* Survival of mysids is one of the most sensitive endpoints in assessment of effects of anthropogenic materials (Cripe and Cripe 1990). Mysids are easily obtainable and have a short life cycle, which make them useful for examination of the additional endpoints of growth and reproduction in a short amount of time (USEPA 1985,1992). Because they are cannibalistic, mysids must be fed during testing and a variety of food rations have been used (ASTM 1996; USEPA 1985,1992) In a previous study, Cripe et al. (1989) demonstrated that in four days, insufficient food can significantly decrease the survival of mysids during exposures to chemicals.

The study described here examined effects on mortality, growth, reproduction, and behavior of *Americamysis bahia* exposed under extended static conditions to bedded sediments from Corpus Christi Bay (Carr et al. 1998).

Correspondence to: G. M. Cripe

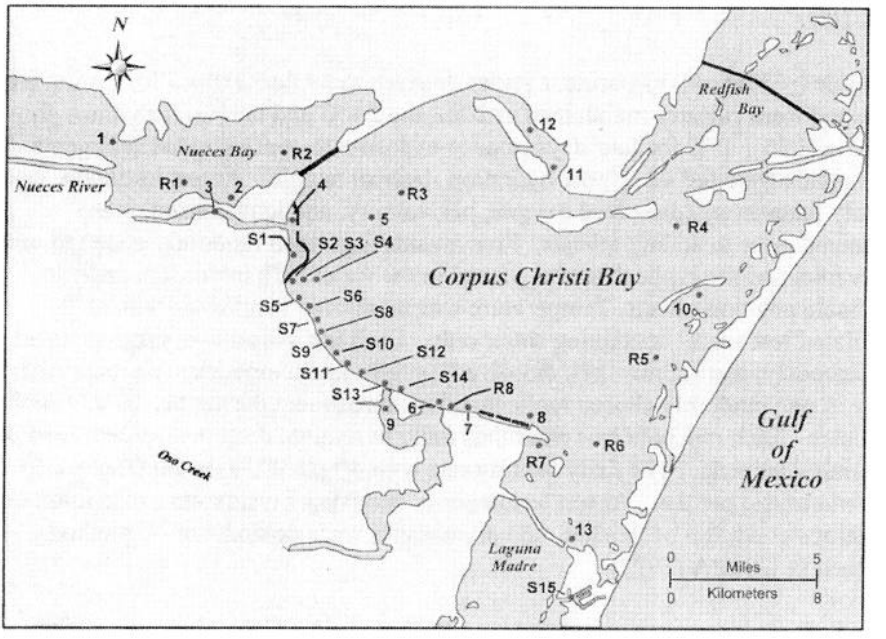

Figure 1. Location of sampling sites in the Corpus Christi Bay National Estuary Program Study (CCBNEP) area (Car-r et al. 1998).

MATERIALS AND METHODS

Thirty-six sediment samples and a control sediment were collected from Corpus Christi Bay to a depth of 8cm ± 2 (SE)(Figure 1). The composite samples were homogenized and stored in one gallon, high density polyethylene containers. The samples were shipped on ice, received within 24 hr at the Gulf Ecology Division of the Environmental Protection Agency, Gulf Breeze, FL and maintained at 4°C for 5 to 18 days prior to use in exposure trials. Subsamples of these sediments were analyzed for total organic carbon, sediment particle composition and contaminants and these analyses have been reported previously (Carr et al. 1998).

One day prior to use in toxicity tests, 100 mL of homogenized sediment were added to each of eight replicate glass 600-mL beakers. Due to the large number of sediments, they were divided into five groups for testing. A set of control sediment replicates (8) were included with each test group. Using a glass plate attached to a glass rod to minimize suspension of the sediments, 400 mL of clean 20‰ salinity seawater were added to the beakers. This water had been filtered to 20μm and aerated prior to use. The beakers were capped with a glass lid through which a glass aeration line extended to within 2-3 cm of the sediment surface. A water bath was used to maintain temperature of test beakers. The beakers were incubated in the water bath with gentle aeration for 24 hr at 26 ± 1 °C prior to adding test animals. The photoperiod for holding and exposures of mysids was

14L/10D.

Juvenile (≤ 24hr old) estuarine mysids *(Americamysis bahia)* used for testing were obtained from cultures maintained at 20‰ and 25 °C and held in flow-through aquaria at 26 ± 1 °C for four days prior to exposure to contaminated sediments. All mysids were fed 48 hr post-hydration *Artemia* nauplii. At test initiation, water quality parameters (dissolved oxygen, pH, salinity, and temperature) were measured prior to adding mysids. Five mysids were then randomly added to each test vessel and the replicates were placed in the water bath in random order to eliminate position effects. Temperature was monitored continuously in a simulated test vessel containing water only. Each test vessel was gently aerated continuously, and salinity, pH, dissolved oxygen, and temperature were monitored daily in one randomly chosen replicate of each treatment during the 10-day static exposure. Each replicate was examined daily to remove dead mysids and feed the remaining animals. The daily feeding rate was 40 (Day l-3) or 60 (Day 4-10) *Artemia*/mysid per day. At test termination, surviving mysids were examined for ovigerous condition of females, and dry weights were determined by methods outlined in USEPA (1992).

Statistical analyses were calculated by a PC based SAS (1996) program. Within groups of sediments tested simultaneously, significant differences in dry weights were determined by ANOVA and Duncans Multiple Range Test at $p \leq 0.05$. Correlation coefficients were calculated to compare sediment composition and mysid growth.

RESULTS AND DISCUSSION

Throughout the testing, a limited number of *Artemia* was provided to each replicate to minimize accumulation of ammonia and effects on dissolved oxygen while maximizing the survival and growth effects in the static, unrenewed 10-day exposure. The food ration was sufficient to produce control growth appropriate for reproducing mysids, as defined in USEPA (1992).

Water quality during the sediment exposures was relatively stable. In all of the 36 sediments and 5 control sediments, mean dissolved oxygen in the overlying water ranged from 70 to 99% saturation throughout the test. Test temperatures were 26 ± 1 °C, mean 10-day salinity ranged from 21 to 24‰, and pH ranged from 7.5 to 8.7.

Analyses of sediments, reported in Carr et al. (1998), indicated several sites with high concentrations of various classes of compounds (Carr et al. 1998). S1 contained arsenic, lead, aluminum, copper, nickel, zinc, cadmium and chromium at concentrations higher than any other site in the study. High molecular weight PAHs were an order of magnitude greater at S9 than the next highest concentrations found at Sl, S2 and site 8. The highest PCBs were found at S14 and elevated also at Sl and S9; DDTs were highest at S9, S2 and Sl and site 8;

and chlordanes were found at S9, S1, S8 and S2. Reference sediments contained only minimal amounts of aluminum, iron, zinc, cadmium and chromium. They generally contained no detectable amounts of PAHs,PCBs or pesticides.

Indications of contamination levels in these sediments can be observed by the Probable Effects Level (PEL) Index (Table 1; Carr 1998). PEL values are the concentration of a chemical above which biological effects are likely to occur. For each site, bulk sediment chemistry concentrations for each of 34 chemicals or class of chemicals was divided by its PEL value and resulting quotients were summed, divided by 31 (the number of PEL values used) and multiplied by 100 to calculate a PEL Index. Sites with greatest contamination have greatest PEL indices. The PEL Index for S9 is substantially higher, perhaps because it contains an order of magnitude greater PAHs and three times the chlorodane than any other site.

Based on the biological endpoints used in this study, this type of whole-sediment exposure is feasible with mysids. Mysid survival in all whole-sediment exposures was excellent, averaging 96.5 ± 3.6%.

Growth is often a more sensitive indicator of long term impacts on a population which increases its value in toxicity screening. Because of the opportunistic nature of mysids, assessment of this endpoint becomes complex when food is limited. Growth of mysids in many of the sediments tested here was significantly different (ANOVA, $p \leq 0.05$) from the control responses (Table 1). These effects seemed to be related to the physical quality of the sediments. Growth data were ranked by clay content of sediment samples (Table 1). Sixteen of the 18 sediments containing ≥7.5 % clay reduced the growth of mysids. Mysids exposed to sediments containing ≤7.5 % clay, with one exception, were either of similar size or larger than those exposed to the control sediment. Statistical analyses indicated a correlation between clay and mean weight of $r = -0.67$ with a significance level of $F = 0.001$. Generally, increased amounts of sand enhanced growth. The difference in growth may be a response to desorption of chemicals from the clay particles or the improved nutritional value of sandy sediments colonized by naturally occurring diatom communities.

Behavioral observations of mysids during this study may provide some insight into the growth differences observed among sediments. During the tests, mysids were observed to collect sediment (predominantly sand grains), manipulate it at the mouth region and drop it. This activity was never observed in sediments of a highly viscous nature. This suggests that auxiliary food was available in some sediments, thus enhancing growth beyond any inhibition that may have resulted from contamination. Another possible explanation for the enhanced growth in the sandy sediments is a hormetic response to either chemical or physical factors. Increased feeding activity has been observed for *M. palmyra* during exposure to aqueous fractions of drilling muds (Carr et al. 1980). An increased food ration with periodic renewal of the overlying water, to reduce ammonia accumulation,

might be required to eliminate the confounding effect of sediment nutrition on contaminant-induced growth effects on mysids during toxicity tests.

Contaminant effects on mysid reproductive processes must be evaluated through exposure during critical developmental stages leading to and including appearance of eggs in the oviducts as described in USEPA (1992). Under the conditions reported here, the fecundity endpoint was not applicable, since eggs were not present in the oviducts of control females at test termination (age, 14 days). The importance of reproductive effects on epibenthic estuarine crustaceans such as mysids is significant, since they are members of the dominant secondary producers in estuaries. The test described here was an initial attempt to quantify effects of estuarine sediments on reproduction, as well as other endpoints. The test lasted 10 days to permit comparison of results to responses of benthic organisms presently used in standardized sediment toxicity tests (Swartz et al. 1997, Breteler et al. 1989, DeWitt et al. 1992). To examine the reproductive response in *A. bahia,* it is recommended that mysids be held for at least 5 days post-release prior to the 10-day exposure for effects assessment.

Experiments of a similar design, utilizing a burrowing amphipod, *Ampelisca abdita* , have been used to characterize a variety of estuarine areas. This species indicated toxicity in 6 of 90 sediments tested from Tampa Bay, FL (Carr, et al. 1996), one of 5 tested from San Francisco and Tomales Bays, CA (Long et al. 1990), one of 36 tested from Corpus Christi Bay, TX (Carr et al. 1998) and 10% of the sediments in the EMAP- Louisiana Province study (Macauley, et al. 1994). In addition, survival of mysids was affected by 5% of the sediments from the Louisiana Province and by none of the Corpus Christi Bay sediments. Limiting assessment of biological impact effects of estuarine sediments to responses of these two species could very likely result in an incorrect conclusion that there are little adverse impacts to the habitability of Corpus Christi Bay sediments. A suite of test organisms utilizing the sediment habitats would likely provide a more accurate evaluation of estuarine sediments.

Table 1. Summary of mysid growth (mg dry weight), PEL Index, total organic carbon (% dry weight), and sediment composition (%) from 10-day exposures of *Americamysis bahia* to 36 Corpus Christi Bay sediments. Mean dry weights are listed with standard deviations in parentheses for all mysids from each exposure.

Site	Growth	Effect[1]	PEL Index[2]	TOC[2]	Sand[2]	Silt[2]	Clay[2]
Control	0.24 (0.01)	-	1.33	0.14	99.4	0.1	0
Control	0.24 (0.02)	-	1.33	0.14	99.4	0.1	0
Control	0.27 (0.02)	-	1.33	0.14	99.4	0.1	0
Control	0.22 (0.02)	-	1.33	0.14	99.4	0.1	0

Table 1. (Continued)

Site	Growth	Effect[1]	PEL Index[2]	TOC[2]	Sand[2]	Silt[2]	Clay[2]
Control	0.27 (0.02)	-	1.33	0.14	99.4	0.1	0
S5	0.30 (0.03)	E	1.74	0.16	99.7	0.3	0
S6	0.36 (0.05)	E	3.53	0.20	99.8	0	0.2
S2	0.28 (0.03)	R	25.85	0.21	98.7	1.0	0.3
S7	0.36 (0.04)	E	2.36	0.19	99.3	0.2	0.4
S8	0.34 (0.01)	E	5.28	0.22	98.4	.5	1.2
S9	0.27 (0.02)	ND	249.64	0.38	93.7	4.9	1.4
2	0.22 (0.05)	ND	2.49	0.11	93.1	5.4	1.5
11	0.33 (0.02)	E	1.60	0.10	97.6	0.8	1.6
S13	0.32 (0.02)	E	1.14	0.11	97.5	0.67	1.8
13	0.39 (0.03)	E	0.95	0.07	96.7	1.4	1.9
R6	0.26 (0.03)	ND	2.10	0.03	97.5	0.21	2.3
S10	0.36 (0.03)	E	1.34	0.16	97.5	0	2.5
7	0.37 (0.02)	E	1.16	0.10	96.6	0.5	2.8
S14	0.47 (0.05)	E	5.75	0.11	96.3	0.7	3.0
S12	0.28 (0.4)	E	1.20	0.13	96.4	0.2	3.3
10	0.39 (0.11)	E	1.18	0.12	95.9	0.4	3.8
S3	0.19 (0.02)	ND	4.09	1.86	93.5	2.0	4.5
S11	0.33 (0.05)	ND	1.10	0.11	91.1	3.3	5.6
R5	0.18 (0.03)	R	2.29	0.18	90.3	2.2	7.5
12	0.21 (0.02)	ND	3.03	0.14	82.5	6.3	11.1
9	0.17 (0.04)	R	3.01	0.44	78.8	9.6	11.6
8	0.15 (0.02)	R	21.34	0.27	74.0	12.8	13.2
3	0.19 (0.02)	R	5.06	0.23	76.6	6.5	17.0
S15	0.16 (0.03)	R	2.62	0.46	71.8	10.3	17.9

Table 1. (Continued)

Site	Growth	Effect[1]	PEL Index[2]	TOC[2]	Sand[2]	Silt[2]	Clay[2]
R2	0.18 (0.02)	R	6.43	0.3	63.5	11.4	25.0
4	0.18 (0.02)	R	7.48	0.4	61.6	13.2	25.2
6	0.17 (0.01)	R	4.97	0.45	64.6	10.1	25.3
R4	0.17 (0.03)	R	4.71	0.46	60.6	12.2	27.1
R7	0.13 (0.03)	R	5.96	0.73	36.2	33.8	30.0
1	0.17 (0.03)	R	6.90	0.57	42.0	27.1	30.9
R8	0.28 (0.01)	E	2.52	0.34	57.8	10.9	31.3
S4	0.18 (0.04)	R	6.12	0.74	48.3	19.5	32.2
R1	0.13 (0.01)	R	9.14	0.71	18.5	39.2	42.3
5	0.17 (0.04)	R	5.79	0.43	36.5	19.4	44.1
S1	0.17 (0.02)	R	39.30	1.66	5.1	43.5	51.4
R3	0.13 (0.02)	R	7.39	0.84	9.68	34.0	56.3

[1]E = Enhanced growth, R = Reduced growth, ND = Not different from control response, ANOVA ($p \leq 0.05$).
[2]From Carr et al. 1998. Greater PEL Index indicates high contamination at the site.

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