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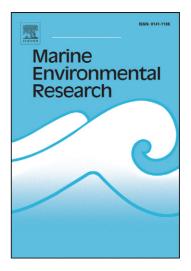
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1	A Comparison of Acute and Chronic Toxicity Methods for Marine Sediments
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22	Abstract. Sediment toxicity tests are valuable tools for assessing the potential effects of
23	contaminated sediments in dredged material evaluations because they inherently address
24	complexity (e.g., unknown contaminants, mixtures, bioavailability). Although there is a need to
25	understand the chronic and sublethal impacts of contaminants, it is common to conduct only
26	short-term lethality tests in evaluations of marine sediments. Chronic toxicity methods for
27	marine sediments have been developed but the efficacy of these methods is less documented. In
28	this evaluation of marine sediments collected from the New York / New Jersey (NY/NJ) Harbor,
29	three 10-day acute toxicity test methods (Ampelisca abdita, Leptocheirus plumulosus,
30	Americamysis bahia) and three chronic and sublethal test methods (28-d L. plumulosus, 20- and
31	28-d Neanthes arenaceodentata) were applied by three testing laboratories. Although the N.
32	arenaceodentata and A. bahia tests did not indicate significant toxicity for the sediments tested
33	in this study, these methods have been reported useful in evaluating other sediments. The 10-d
34	A. abdita, 10-d L. plumulosus and 28-d L. plumulosus tests were comparable between
35	laboratories, indicating $29 - 43\%$, 29% , and $43 - 71\%$ of the tested sediments as potentially
36	toxic. The 28-d L. plumulosus method was the only chronic toxicity test that responded to the
37	test sediments in this study. The 28-d L. plumulosus endpoint magnitudes were related to
38	sediment chemistry and the sublethal endpoints were reduced as much or more than acute
39	lethality endpoints. However, intra-treatment sublethal endpoint variability was greater,
40	compromising detection of statistical significance. In this study, the chronic L. plumulosus test
41	method was less consistent among laboratories relative to acute test methods, identifying
42	potential for toxicity in a similar number (or slightly more) NY/ NJ Harbor sediments.
43	Keywords - Sediment toxicity, bioassay, chronic, sublethal, amphipod, polychaete

44 **1. Introduction**

45 More than 55 million cubic yards of sediment are dredged from harbors and channels 46 annually in the United States and placed in the ocean. Of this material, it is estimated 47 approximately 2.5 million cubic yards require special management because of chemical 48 contamination (U.S. ACE, 2008). Sediment can be placed in the ocean if it is determined that 49 the material does not "... unreasonably degrade or endanger: human health, welfare, or amenities, 50 marine environment, ecological systems, or economic potentialities" (Marine Protection Research and Sanctuaries Act of 1972). A risk-based chemical and biological evaluation must 51 52 be conducted to determine suitability for ocean placement of dredged sediments (U.S. EPA/U.S. 53 ACE, 1991). This assessment includes an evaluation of direct toxicity of sediments to benthic 54 aquatic invertebrates.

Whole-sediment toxicity tests are commonly used in contaminated site assessments and 55 56 dredged material evaluations. Several organizations (U.S. Environmental Protection Agency 57 (U.S. EPA), American Society for Testing Materials (ASTM), Environment Canada (EC) and the 58 International Standards Organization (ISO)) have published test methods to specifically evaluate 59 the toxicity potential of freshwater (e.g., U.S. EPA, 2000; ASTM, 2005) or marine (e.g., U.S. 60 EPA, 1994; ASTM, 2000; ASTM, 2003) sediments. Toxicity tests can be conducted for acute or 61 chronic exposure durations. Acute tests typically assess mortality over short-term exposures, 62 although some freshwater (U.S. EPA, 2000; ASTM, 2005) and marine and estuarine (Moreira et al., 2005) sediment tests also assess sublethal endpoints. Chronic tests represent a larger portion 63 64 of the organism's life cycle and directly address sublethal endpoints (e.g., growth, reproduction) 65 in addition to survival.

66	It is common for evaluations of marine sediments to employ only acute benthic toxicity
67	test methods. Exceptions include use of a 20-day (-d) chronic polychaete test method (Johns et
68	al., 1990) in the Dredged Material Management Program (DMMP) and consideration of several
69	chronic methods in southern California (Bay et al., 2007). Given that acute tests for marine
70	sediments may not be predictive of population-level effects, there is support for the use of
71	chronic test methods (Munns et al., 2002; Wenning et al., 2005). These longer exposure tests
72	that directly measure sublethal endpoints may provide information amenable for population
73	modeling (McGee and Spencer, 2001; Ingersoll et al., 2005). Additionally, Finkelstein and
74	Kern (2005) presented evidence to suggest that acute tests may result in more false negatives
75	(i.e., failure to identify toxic sediments) compared to chronic methods when interpreted relative
76	to sediment chemistry data expressed as effects range medium quotients. Chronic, or sublethal,
77	sediment toxicity methods for estuarine and marine sediments have been published in guidance
78	documents (e.g., ASTM, 2000; Johns et al., 1990; U.S. EPA/ACE, 2001) and in peer-reviewed
79	journals (e.g., McGee and Spencer, 2001; Heuvel-Greve et al., 2007). Although chronic test
80	endpoint sensitivity for freshwater sediment method has been studied widely (Ingersoll et al.,
81	2001), the effectiveness of chronic test methods for marine sediments is less known.
82	Other comparisons of marine and estuarine sediment toxicity test performance have been
83	conducted between single (Mearns et al., 1986; Bay et al., 2003) and multiple (Schlekat et al.,
84	1995; Casado-Martinez et al., 2006) acute test methods, single chronic test methods (Johns et al.,
85	1990; U.S. EPA/ACE, 2001) and acute and chronic test methods that use the same (McGee et al.,
86	2004; Kuhn et al., 2002; Castro et al., 2006; Gale et al., 2006) or different (Anderson et al., 1998;

87 Pinza et al., 2002; Greenstein et al., 2008) test organisms. The overall goal of this study was to

88 conduct an interlaboratory comparison of a suite of acute and chronic test methods relevant to

89 evaluate New York / New Jersey (NY/NJ) Harbor (USA) sediments that are applicable to a 90 dredged material management program that involves annual dredging of more than five million 91 cubic vards of sediment for navigation and construction projects. The U.S. EPA requires 10-d 92 acute Ampelisca abdita and Americamysis bahia bioassays as one line of evidence for the North 93 Atlantic region to determine the suitability of sediments for ocean placement. We conducted 94 tests and compared results between these currently applied acute sediment toxicity tests to an 95 alternative acute test method (10-d Leptocheirus plumulosus) and available chronic toxicity test 96 methods (28-d L. plumulosus and 20-d, 28-d N. arenaceodentata) to determine relative utility to 97 this region-specific dredged material management program. Specific objectives were to investigate (1) the relative test method performance, (2) the consistency of results among three 98 99 laboratories, and (3) whether the chronic tests offered an enhanced level of sensitivity to 100 contaminants in the tested sediments. 101 2. Materials and Methods 102 2.1. Study design 103 This study involved preliminary and interlaboratory evaluations of sediments collected 104 from the New York / New Jersey (NY / NJ) Harbor (Figure 1). Sediment aliquots were analyzed 105 for contaminants on separate occasions because of a six-month storage period that exceeded the 106 recommended eight-week holding time for sediments. Traditional holding time 107 recommendations were not met because of the need for a preliminary assessment of the sediment 108 toxicity prior to the interlaboratory comparison. However, holding time requirements were not 109 directly applicable since the objective of this study was to compare the relative responses of 110 different test methods to sediments rather than to assess the potential for site toxicity. The 111 preliminary evaluation (December 2003 to February 2004) was conducted at only Lab A to

112 evaluate sediments from all nine sample sites. The toxicity tests conducted were the acute (10-d) 113 A. abdita, A. bahia, and L. plumulosus and the chronic (28-d) L. plumulosus and 28-d N. 114 arenaceodentata test methods. Additionally, control sediment (full description provided in 115 Section 2.2) was used to dilute the Newark (100, 77, 50, 35, 24% dry weight) and Hudson (100, 116 82, 59, 43, 31% dry w/w) sediments using the 10-d L. plumulosus test to assess toxicity. 117 The interlaboratory evaluation (August – September 2004) involved three labs 118 experienced in sediment toxicity testing (see author affiliations). Four distinct sediments of 119 varying contamination (Hudson, Chester, Red Hook, and Newark) and a Newark dilution series 120 (75%, 50%, 25% dry w/w) were selected based on preliminary results from the nine collected 121 sediments. Methods used were the acute A. abdita test, chronic L. plumulosus, and three 122 different N. arenaceodentata test methods summarized in Table 1. The 10-d A. bahia test was 123 excluded because of low overall response in the preliminary evaluation. An acute L. plumulosus test was initiated at one laboratory (Lab A) on the same day as the chronic L. plumulosus tests. 124 Each test method was initiated on the same day at the three facilities using organisms from the 125 126 same sources. 127 [Table 1 position]

128 2.2. Test Sediments

Nine sites (Figure 1) in the NY/NJ Harbor were selected based on varying historic levels
of contamination. Sediment was collected (27 – 29 September 2003) using a 10-L Van Veen
grab and stored in 20-L HDPE buckets according to guidance (U.S. EPA, 2001). Buckets were
shipped overnight (4 ± 2 °C) to the U.S. Army Engineer Research and Development Center
(ERDC, Vicksburg, MS, USA). A reference sediment located south of New York Harbor (40°
20.210' N, 73° 52.190' W), designated for testing by the U.S. EPA Region 2 dredging program,

135	was acquired by Aqua Survey, Inc (Flemington, NJ, USA). A control sediment was collected
136	from Sequim Bay, WA (Battelle, Sequim, WA, USA, 48° 06.52' N, 123° 01.410' W). All
137	sediments were thoroughly homogenized to consistent texture with an impeller mixer before use.
138	[Figure 1 position]
139	2.3. Sediment chemistry
140	Chemical analyses followed U.S. EPA SW-846 methodology (unless otherwise
141	specified). The homogenized sediments were analyzed for pesticides, polychlorinated biphenyls
142	(PCBs), polycyclic aromatic hydrocarbons (PAHs), total metals, simultaneously extracted metals
143	(SEM), and acid volatile sulfides (AVS) by the ERDC Environmental Chemistry Branch.
144	Samples were submitted to Severn Trent Laboratories (Knoxville, TN) to analyze dioxins,
145	furans, alkyl tins (GC-MS; method 8270), and sediment grain size (ASTM D422). To determine
146	ammonia concentrations, sediment porewater was extracted by 15-minute centrifugation of
147	sediment at 2,700 g and analyzed using a 720A ion selective electrode meter, equipped with a
148	95-12 ammonia-sensitive electrode (Thermo Orion Electron Corp.).
149	2.4. Sediment toxicity tests
150	Three acute toxicity tests and three chronic toxicity test methods were conducted (Table
151	1). The A. abdita and L. plumulosus tests were conducted according to standard guidance (U.S.
152	EPA, 1994) while the A. bahia sediment test followed Appendix E of the Inland Testing Manual
153	(U.S. EPA/ACE, 1998), since no U.S. EPA or ASTM sediment testing methods were available.
154	The chronic <i>L. plumulosus</i> survival, growth, and reproduction test followed U.S. EPA / U.S.
155	ACE (2001) while the three chronic <i>N. arenaceodentata</i> followed various methods (ASTM,
156	2000; Johns et al., 1990; Bridges et al., 1997). Ampelisca abdita was field-collected from the

157 Blackwater River at Sea Brook, NH, USA (Aquatic Research Organisms, Hampton, NH) for the

158 preliminary comparison or from Atlantic Highlands, NJ, USA (Aqua Survey, Inc.) for the 159 interlaboratory comparison. The remaining three organisms were laboratory-reared (A. bahia; 160 obtained from Applied Biosystems (Fort Collins, CO, USA); L. plumulosus obtained from 161 ERDC in-house cultures; N. arenaceodentata were obtained from Don Reish (California State 162 University-Long Beach, CA, USA)). Since the concern of this study was endpoint response 163 related to persistent contaminants rather than ammonia, sediments were purged for 48 h 164 according to U.S. EPA (U.S. EPA, 1994) prior to test organism addition to a more conservative level of 20 mg/L for interstitial water (Ferretti et al., 2000). Ammonia concentrations were 165 166 determined in separate test chambers not used in tests. Criteria governing test acceptability included a method-specific minimum control survival value (Table 1) and water quality within 167 168 prescribed limits (U.S. EPA, 1994; Johns et al., 1990; U.S. EPA/ACE, 2001). The overlying 169 water quality (temperature, pH, salinity, dissolved oxygen) was measured daily.

170 2.5. Statistical analysis

171 Tests were conducted in a randomized and blind fashion. Data normality (Kolmogorov-172 Smirnov test), homogeneity (Levene's Test), and treatment differences compared to the control 173 sediment (one way ANOVA and Dunnett's Method, two way ANOVA and Tukey's test) were 174 determined at the $\alpha = 0.05$ level using SigmaStat software (SPSS, Chicago, IL). Survival data 175 were arcsine-square-root transformed and sublethal endpoints were square-root transformed 176 when necessary for normality. When normality could not be achieved, the Kruskal-Wallis one-177 way ANOVA on ranks was applied. Since the study objective was to compare relative test 178 method performance, not to characterize toxicity at a site, the control sediment was used in 179 statistical comparisons rather than the site reference sediment (comprised only of 2% fines), 180 which did not have representative characteristics of the predominantly fine test sediments. In

181 addition to statistical significance compared to the control, a toxicological decision criterion 182 (TDC), defined as a reduction in survival that was at least 10% (20% for amphipod lethality) less 183 than that of the control (U.S. EPA/ACE, 1998), was applied. For survival, both statistical 184 significance and the TDC were required to classify sediments as potentially toxic, while only a statistically detectable decrease relative to the control was needed for sublethal endpoints. The 185 186 lethal median concentration producing 50% mortality (LC50) in sediment dilutions was determined by the Spearman-Karber method using Toxstat[®] software (Gulley 1996, University 187 188 of Wyoming). 189 To assess test endpoint performance, four metrics were calculated: (1) the response 190 magnitude (RM), (2) the minimum significant difference (MSD), (3) Kendall's concordance test, and (4) Spearman correlations. The RM estimated the relative amount of response between test 191 192 endpoints due to sediment exposure by taking the grand mean of inter-treatment reductions 193 compared to the control (modified from Gray et al., 1998). $RM = \frac{\frac{1}{k} \sum_{i=1}^{k} |x_i - x_c|}{2} * 100$ 194 (1) 195 Where, k = number of treatments, $x_i =$ treatment mean, $x_c =$ control mean 196 197 The MSD measured the smallest endpoint reduction needed in a sediment treatment for a 198 statistically significant difference relative to the control (Chapman et al., 1995). $MSD = d * s_w \sqrt{\frac{1}{n_o}} + \sqrt{\frac{1}{n_o}}$ 199 (2) 200 d = critical value for Dunnett's procedure, s_w = square root of within mean square, n_0 = treatment replicates, n_c = control replicates 201 202 203 Calculations of the RM and MSD were expressed as a percentage of the control mean to 204 facilitate comparison between lethal and sublethal endpoints. Treatment endpoint values greater 205 than control values were adjusted to the control value. Kendall's concordance test (W) was used

to assess interlaboratory agreement between ranks of endpoints to examine the consistency of

207	tests (U.S. EPA/ACE, 2001; Mearns et al., 1986; Bay et al., 2003; Schlekat et al., 1995; Zar,
208	1984).
209	3. Results
210	3.1. Sediment chemistry and characterization
211	A summary of physical and chemical characteristics is provided in Table 2. Most
212	sediments consisted of greater than 90% fines, with some exceptions. Reductions in PAH
213	concentrations during storage were observed in the sediments for the interlaboratory evaluation.
214	A more detailed summary of analytical chemistry data and correlation analysis is reported in
215	Steevens et al. (2008).
216	[Table 2 position]
217	3.2. Sediment toxicity tests
218	Water quality parameters were within test method specified ranges, with the exception of
219	one overnight temperature deviation (17.9 °C) in one chronic L. plumulosus test in the
220	interlaboratory evaluation (Lab A) that was quickly rectified and did not appear to impact
221	organism condition. Total porewater ammonia concentrations (following purging) at test
222	initiation and termination were below recommended thresholds (< 20 mg/L) in all tests. In
223	general, test endpoints (e.g. survival, reproduction) in the reference sediment (comprised of 98%
224	sand) were reduced relative to the control sediment in the acute and chronic L. plumulosus tests
225	but were variable in the A. abdita tests and similar in the N. arenaceodentata tests relative to the
226	control (Table 3, Figure 2).

227 3.3. Preliminary evaluation

228	Toxicity test results are presented in Table 3. Mean survival in the control met
229	acceptability criteria in all tests, with the exception of the A. abdita test (87%). In the A. bahia
230	test, mean survival was relatively high (> 75%), with no statistically significant differences
231	among sediment treatments. The 28-d N. arenaceodentata test also resulted in no significant
232	survival (range: $60 - 100\%$) or growth (Table 3) reduction effects for any sediment. Significant
233	reductions in test endpoints were observed for the acute (A. abdita, L. plumulosus) and chronic
234	(<i>L. plumulosus</i>) test methods using amphipods.
235	In a separate 10-d L. plumulosus test (data not presented), a concentration-dependent
236	relationship was observed for dilutions series of the Hudson and Newark sediments, with 10-d
237	LC50 values of 32 (22 – 46, 95% CIs) % and 48 (43 – 54, 95% CIs) %, respectively. This
238	testing was used to select a sediment for dilution in the interlaboratory comparison. Also,
239	survival in the undiluted Hudson ($12 \pm 12\%$) and undiluted Newark ($20 \pm 11\%$) treatments were
240	similar to those obtained from previous testing (compare to Table 3).
241	[Table 3 position]

242 3.4. Interlaboratory evaluation

The test sediments were stored for 11 months (at 4 °C) after collection, 6 months following the preliminary evaluation, prior to use in the interlaboratory evaluation. Reductions in PAH concentrations (Table 2) and increases in test organism survival were observed (Table 3 and Figure 2). Interestingly, slight but consistent increases in PCBs, dioxins and DDTs were observed in the sediments following storage. This did not influence the use of the sediments for relative comparison between test sensitivity, which was the objective of the study. Two tests were excluded because of failures to meet control acceptability criteria; 10-d *A. abdita* control

survival was less than 90% (Lab A) and 28-d *L. plumulosus* failed to meet the control survival (<
80%) and reproduction acceptability criteria (Lab C).

For the two acceptable *A. abdita* tests, statistically significant survival reductions greater
than the TDC were recorded (Figure 2). Two-way ANOVA determined no significant
differences between laboratories after allowing for variability that was due to sediment effects,
although individual significant differences in survival were determined between laboratories for
the Reference and Chester sediments.

In contrast to the preliminary evaluation, no statistically significant reductions in survival 257 258 were observed among sediments for the two acceptable 28-d L. plumulosus tests (Figure 2). 259 However, there were statistically significant differences in survival between the two laboratories for the Reference, Red Hook and 50% Newark sediments. Significant reductions in either 260 biomass or reproduction were observed for five and three (out of seven) sediments. After 261 262 factoring out variability between the two laboratories by using two-way ANOVA, significant 263 reductions in the biomass and reproduction endpoints were observed in both tests for the Reference, Hudson and 100% Newark sediments; reproduction was significantly reduced in 75% 264 265 Newark sediment.

As in the preliminary study, survival was high in all chronic *N. arenaceodentata* tests, with no statistically significant endpoint reductions. Significant increases in biomass occurred in some sediments (Figure 3). Overall biomass was statistically significantly greater (four-fold) in the 20-d DMMP protocol compared to the other two methods that used lower feeding rations. Biomass was generally determined to be significantly greater in the Lab B tests by two-way ANOVA. Mean variability was comparable for the 28-d (coefficient of variation (CV) = $30 \pm$

272	16%) and the 20-d (CV = $30 \pm 18\%$) test methods and slightly lower for the modified 20-d te	est
273	method (CV = $24 \pm 12\%$).	

274 The RM, a measure of sensitivity, and MSD, a measure of statistical power, values are 275 graphically summarized for each test endpoint in Figure 4. Test endpoints generally showed 276 greater response in the preliminary evaluation relative to the interlaboratory evaluation. Among 277 test methods, the lowest responses were for interlaboratory 10-d A. bahia, 28-d L. plumulosus 278 survival (Figure 4), and N. arenaceodentata endpoints. The highest response was observed in 279 28-d L. plumulosus reproduction. However, higher statistical power (i.e., smaller MSD) was 280 observed for survival endpoints (acute and chronic) than for sublethal endpoints. Among 281 sensitive sediment toxicity tests, the response to test sediments was similar for individual test 282 methods with good overall agreement between ranks of endpoint magnitudes. Kendall's concordance test (W) determined high coefficients of agreement for A. abdita survival (W = 283 284 0.85, p < 0.25), L. plumulosus survival (W = 0.80, p < 0.25), L. plumulosus biomass (W = 0.77, p 285 < 0.25), and L. plumulosus reproduction (W = 0.95, p < 0.10). Lower significance was because 286 of test failures (n value reduced from three to two tests). 287 [Figure 2 position]

- 288 [Figure 3 position]
- [Figure 4 position]
- 290 **4. Discussion**

The overall objective of this study was to compare the performance of acute whole sediment toxicity tests commonly used in evaluations of NY/NJ harbor sediment to alternative chronic methods discussed in regulations but not commonly applied in practice. Varying endpoint response to the sediments evaluated was observed among test methods, ranging from

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295 low response (acute Americamysis bahia, chronic Neanthes arenaceodentata) to moderate 296 response (acute Leptocheirus plumulosus and Ampelisca abdita survival) to high response 297 (chronic *L. plumulosus* reproduction) (Figure 4). Relative differences in endpoint response has 298 been previously reported among test methods, testing facilities, evaluations, and sediments with 299 differing predominant contaminants (Bay et al., 2007; Mearns et al., 1986; Bay et al., 2003; 300 Kuhn et al., 2002). Generally, no one species or test method is universally most sensitive across 301 sediments and conditions, supporting guidance recommending the use of multiple species to 302 assess sediment toxicity. 303 Endpoint response to sediments is not necessarily indicative of contaminant effects. Several factors may complicate the interpretation of sediment tests, including uncertainty 304 305 (Vorhees et al., 2002) and confounding factors (e.g., grain size, ammonia, sulfides, indigenous 306 animals) (Postma et al., 2002). Grain size, for instance, is a well-known confounding factor in sediment tests (U.S. EPA, 1994; U.S.EPA/ACE, 2001) that can cause amphipod mortality by 307 308 physical or energetic stress when outside species-specific tolerance ranges (Emery et al., 1997). With the exception of the reference sediment (98% coarse grains), the grain-size distributions of 309 310 sediments tested in this study were similar (Table 2) and unlikely to produce confounding 311 mortality. The different grain size of the reference sediment was not representative of the 312 predominantly fine-grained test sediments and may have caused the reduced L. plumulosus 313 endpoints observed in the reference sediment (Table 3, Figure 2). Porewater ammonia is another 314 stressor in NY / NJ Harbor sediment evaluations that can confound tests if not purged as 315 described in guidance. In this study porewater ammonia was purged to a more conservative 316 value of 20 mg/L to remove it as a confounding factor to focus more on persistent contaminants 317 of concern.

318	While the acute Americamysis bahia test method provided reasonable statistical power
319	(Figure 4), the survival endpoint of this test was not as sensitive to the tested NY/NJ Harbor
320	sediments as the other acute methods examined. This sediment test method is applied to evaluate
321	NY/NJ Harbor sediments to provide a taxon other than amphipods, and it has responded to
322	contaminated sediments in other studies (e.g., Ho et al., 2000). The sensitivity and exposure of
323	A. bahia has been best demonstrated in the water column (U.S. EPA, 2002) and the Inland
324	Testing Manual (U.S.EPA/ACE, 1998) provides the only national guidance for sediment
325	evaluations using this species. This test method supplies an epibenthic exposure to sediments,
326	since A. bahia dwells predominantly in the water-sediment interface, and includes a daily
327	feeding ration (unlike the other acute test methods). The above factors should be considered
328	when the intention is to be protective of infauna that ingest sediments containing hydrophobic
329	contaminants. This method may be useful for test sediments with physical characteristics that are
330	not suitable to infaunal life (e.g., clays).

The chronic *N. arenaceodentata* test endpoints were not responsive to the evaluated NY / 331 332 NJ Harbor sediments. However, others (Anderson et al., 1998; Bridges et al., 1997; Green et al., 333 1999) have reported reductions in N. arenaceodentata endpoints, more often expressed for 334 growth than survival, in exposures to contaminated sediments. The 20-d method is documented 335 in a routine testing program (DMMP). In previous evaluations of test sediments from other 336 regions, 28-d N. arenaceodentata growth responded similarly to 10-d L. plumulosus survival 337 (Farrar, unpublished data). The 20-d N. arenaceodentata test method was reported to outperform 338 the chronic (28-d) L. plumulosus test method (Pinza et al., 2002) and has responded to more 339 (Pinza et al., 2002) and fewer (Anderson et al., 1998) sediments relative to the 10-d acute 340 amphipod *R. abronius* method. The sensitivity of the *N. arenaceodentata* tests may vary

341 remarkably among major chemical classes and may be less responsive to PAHs because of the 342 species' efficient metabolism (Christensen et al., 2002). Although the N. arenaceodentata 343 endpoints were not responsive in this study, differences were observed among methods. The 344 20-d method involved a larger feeding ration and produced larger worms than the 28-d and 345 modified 20-d tests (Figure 3). Bridges et al. (1997) demonstrated that relatively large amounts 346 of supplemental food can reduce the toxicity of test sediments. Also, the 20-d methods use five 347 worms per test chamber (compared to one per chamber in the 28-d method), which may inherently produce greater growth variability because of dominant worm interactions within 348 349 replicates (Anderson et al., 1998; Bridges et al., 1997). Only the test methods employing amphipods resulted in statistically reduced endpoint 350 351 responses in exposure to the tested NY / NJ Harbor sediments, and the 28-d L. plumulosus 352 method was the only chronic test in this sediment-specific evaluation to result in statistically 353 significant reductions in test endpoints (Table 3, Figure 2). Mean percent survival was generally 354 higher in the interlaboratory evaluation, possibly resulting from contaminant loss (e.g., PAHs, Table 2) after extended storage (Norton et al., 1999). Amphipod test methods suggested toxicity 355 356 in at least one endpoint relative to the control for the most highly contaminated sediments (i.e., 357 Hudson, 100% Newark). The presented data set conveys no clear advantage between the acute 358 amphipod test methods in terms of statistical significance (Figure 4). However, logistically L. 359 *plumulosus* offered the following advantages; (1) stronger correlations between survival and 360 sediment chemistry (Steevens et al., 2008), (2) existing laboratory cultures making them 361 available throughout the year; and (3) burrowing activity into sediments without tube-building 362 (U.S. EPA, 1994; Bay et al., 2003; Ho et al., 2000) that provides a clear vector for sediment 363 exposure, and more expedient processing at test termination.

Test endpoint performance was comparable among laboratories for the responsive test methods (i.e., acute *A. abdita*, chronic *L. plumulosus*) based upon good agreement (Kendall's W) between ranks of endpoint magnitudes. Overall, "testing laboratory" was not a significant factor on the 10-d *A. abdita* data but did contribute to significant interlaboratory differences in the 28-d *L. plumulosus* test method (also indicated in Figure 4). Thus, the chronic test method may have been more susceptible to differences among laboratory conditions.

370 The sublethal reproduction endpoint in the chronic L. plumulosus test method offered 371 similar or enhanced response to the tested sediments relative to lethality (Figure 2, Figure 4). 372 However, this magnitude of response is not meaningful in the context of management decisions that apply sediment test data based on presence or absence of statistically significant endpoint 373 reductions relative to the reference (U.S. EPA, 1994; U.S. EPA/ACE, 1998). In this regard, the 374 375 associated statistical power (i.e., MSD) of the L. plumulosus sublethal endpoints in this study 376 was lower than survival endpoints, diminishing the ability of statistical tests to detect differences. 377 The acute A. abdita and L. plumulosus survival endpoints performed more consistently than the 378 sublethal L. plumulosus endpoints (Figure 4) because of high intra-treatment sublethal endpoint 379 variability. Some studies have found enhanced sensitivity in chronic sediment test methods 380 relative to acute methods assessing estuarine and marine sediments using amphipods (Castro et 381 al., 2006), a copepod (Bejarano et al., 2004) and a polychaete (Moreira et al., 2005; Rice et al., 382 1995). However, the results from this study and from previous studies (McGee et al., 2004; 383 Pinza et al., 2002; Greenstein et al., 2008) indicate that the specific chronic sediment toxicity 384 methods we evaluated performed less consistently relative to acute methods.

In addition to confounding factors relevant to all tests (described above), multiple factors
 specific to chronic sediment toxicity test methods for marine sediments may contribute to

387 differences in performance (McGee et al., 2004; Kuhn et al., 2002). The response of different 388 endpoints (e.g., survival vs. growth) may reflect differing modes of action (Kuhn et al., 2002), 389 inherent sublethal endpoint variability (McGee et al., 2004; Pinza et al., 2002), or different 390 procedures applied in the test methods (Table 1). Although intra-treatment variation may be 391 reduced through increased replication or expression of neonates produced as a function of 392 number of sexually mature females, the increased labor of such approaches may not be cost 393 effective (Gray et al., 1998). Also, the addition of supplemental food to the sediment matrix in 394 chronic toxicity test methods for marine or estuarine sediments may reduce chemical 395 bioavailability (McGee et al., 2004) relative to organisms in acute tests that receive no 396 supplemental food (Lotufo et al., 2001). Such differences do not exist between acute and chronic 397 test methods for freshwater sediments, as both employ feeding rations and water exchanges (U.S. EPA, 2000; McGee and Spencer, 2001; Bridges et al., 1997). 398

399 5. Conclusion

400 The results of this study indicated performance differences for several marine sediment 401 toxicity test methods. While the 10-d A. abdita method performed comparably to the 10-d L. 402 plumulosus, the other acute method employed in these evaluations (10-d A. bahia) did not 403 respond to the sediments in this study. Of the chronic test methods investigated for use, only the 404 28-d L. plumulosus method responded to the tested sediments. Advantages and disadvantages of 405 the chronic L. plumulosus method were highlighted for NY / NJ Harbor dredged material 406 evaluations. The potential benefits of the 28-d L. plumulosus test include theoretical 407 improvements in level of protection through direct measure of sublethal effects, large sublethal 408 endpoint response, strong relationships with sediment contamination, use of endpoints needed 409 for population modeling, and fewer false negatives (Finkelstein and Kern, 2005). The

410 drawbacks of the 28-d L. plumulosus test may include, difficulties related to sublethal endpoint 411 variability, longer turn-around time to obtain results, difficulty meeting acceptability criteria 412 (more can go wrong in longer tests), and increased labor intensiveness leading to increased cost. 413 Chronic estuarine and marine sediment toxicity test methods generally cost 1.7 (28-d N. 414 arenaceodentata) to 2.3 (28-d L. plumulosus) times more than acute tests (based on per treatment 415 costs reported by Bay et al., (2007)). The ability of the 28-d L. plumulosus chronic toxicity test 416 method to indicate greater sensitivity to contaminated sediments relative to the 10-d test method was not consistent (McGee et al., 2004; this study). Overall, evidence exists for lower response 417 418 (DeWitt et al., 1996; Fuchsman et al., 1998; Green et al., 1999; McGee et al., 2004) and 419 enhanced response (McGee et al., 1993; Sferra et al., 1999) of L. plumulosus sublethal endpoints. 420 Although the results of this study provided good indication of which test methods (i.e., acute and 421 chronic amphipods tests) have the strongest application to NY / NJ sediments and associated 422 contaminants of concern, more rigorous testing is needed before attempting to generalize to other 423 regions. Test method selection should be contingent on management goals (e.g., desired level of 424 protection, required use of indigenous taxa, desire for estimation of population level effects) and 425 organism suitability to site-specific sediment composition 426

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651 Figure legends

652

Figure 1. Sampling stations in the New York Harbor.

654

Figure 2. Test results for the interlaboratory comparison. Survival results for the 10-d

656 Ampelisca abdita, 10-d Leptocheirus plumulosus and 28-d L. plumulosus test methods are shown

657 in panel (a). Biomass for the 28-d *L. plumulosus* test method is shown in panel (b).

658 Reproduction for the 28-d *L. plumulosus* for the test method is shown in panel (c). Histogram

659 columns indicate mean values and bars represent one standard deviation from the mean.

660 Asterisks represent a statistically significant reduction compared to the control and an endpoint 661 response greater than the toxicological decision criterion (i.e., > 20% reduction relative to the

661 response greater than the toxicological decision criterion (i.e., > 20% reduction relative to the control).

663

Figure 3. *Neanthes arenaceodentata* toxicity test results for the 28-d, 20-d DMMP and 20-d

665 DMMP with modified feeding ration test methods. Histogram columns indicate mean values and

bars represent one standard deviation from the mean. Number signs represent significant

667 increases compared to the control.

668

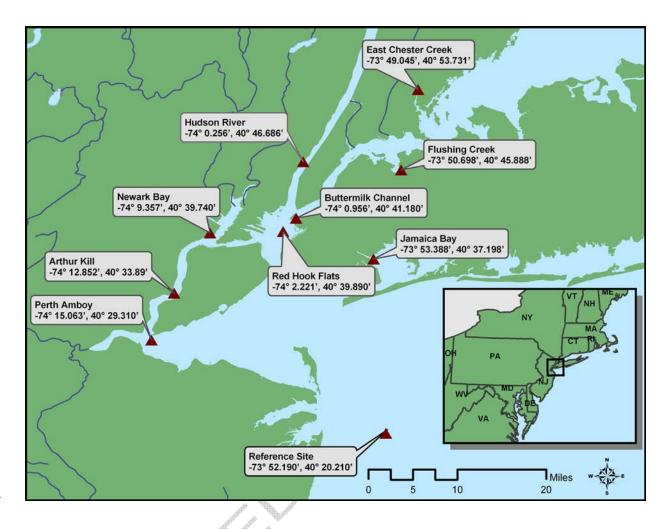
669 **Figure 4.** Plot of the Response Magnitude (RM) relative to the control versus the Minimum

670 Significant Difference (MSD) needed for statistical comparisons for the toxicity test endpoints

evaluated in this study. Data from the preliminary data (open points) and interlaboratory (closed

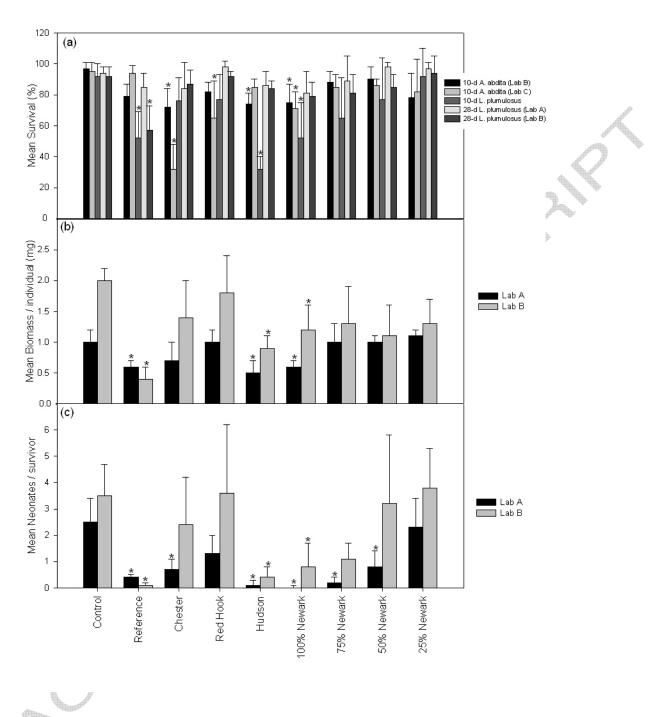
points) are indicated. Dotted vertical lines around points indicate the range in endpoint data

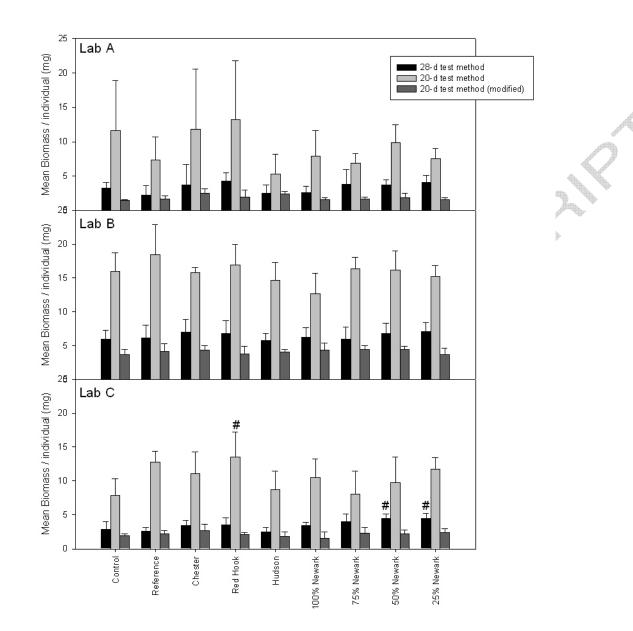
673 while the diagonal dashed line indicates a one-to-one relationship.



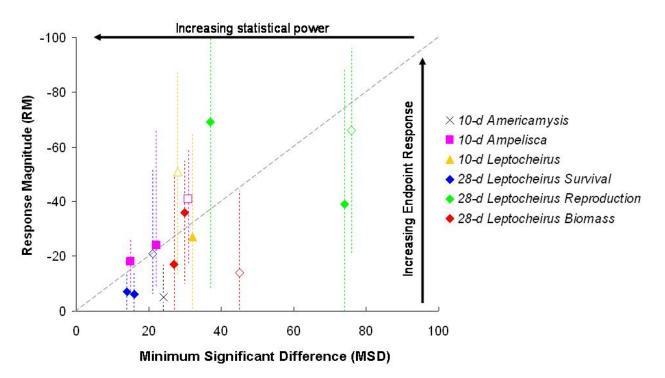
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C) Class



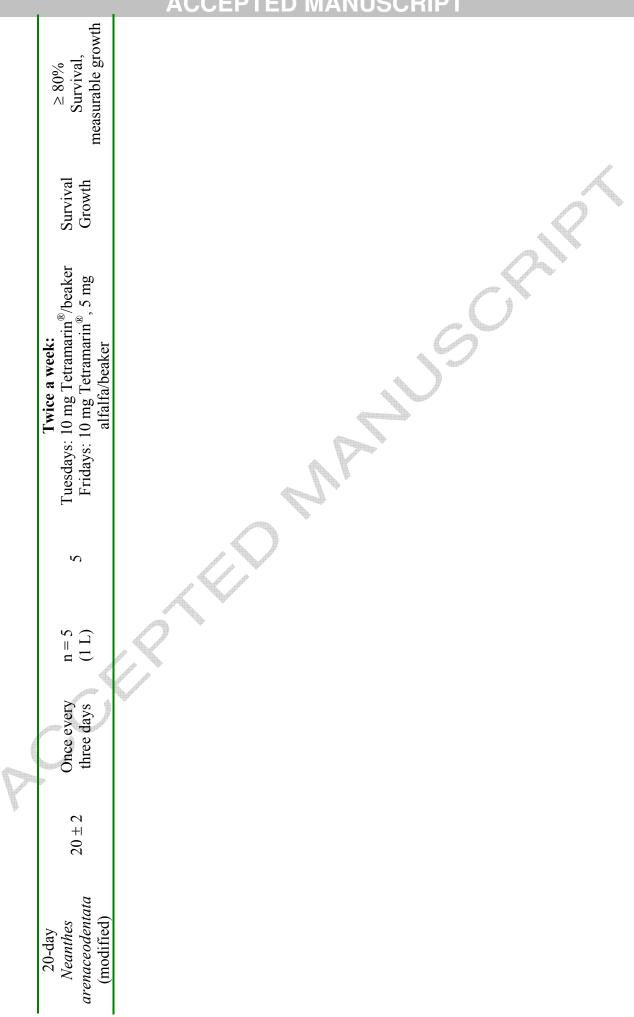


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Test MethodTemperature (°C)Water (°C)Replicates per (°C)Oganisms per (°C)Feeding per (°C)Endpoint(s)Control AcceptabilityControl Acceptability10-day botia 0 ± 2 None $n = 5$ (11)20Antemia nauplii dailySurvival $2 90\%$ survival $2 90\%$ survival $2 90\%$ survival $2 90\%$ survival $2 90\%$ survival $2 90\%$ survival $2 90\%$ survival10-day obtia 20 ± 2 None $n = 5$ (11)20NoneSurvival $2 90\%$ survival10-day obtia 20 ± 2 None $n = 5$ (11)20NoneSurvival $2 90\%$ survival28-day planutosus 25 ± 2 None $n = 5$ (11) $2 0$ NoneSurvival $2 90\%$ survival28-day planutosus 2 ± 2 One $n = 10$ 1 1 NoneSurvival $2 90\%$ survial28-day planutosus 2 ± 2 One $n = 10$ 1 1 1 Survival $2 90\%$ survial28-day submitosus 2 ± 2 One $n = 10$ 1 1 1 1 Survival $2 90\%$ survial28-day submitosus 2 ± 2 One $n = 10$ 1 1 1 1 1 $2 90\%$ survial $2 90\%$ survial29-day submitosus 2 ± 2 0 ± 2 1 1 1 1 1 1 1 1 20-day submitosus 2 ± 2 1	Table 1.	Table 1. Summary of whole sediment toxicity test methods.	ole sediment t	toxicity test me	ethods.				
20 ± 2 None $n=5$ $(1,1)$ 20Artemia mauplii dailySurvival 20 ± 2 None $n=5$ $(1,1)$ 20NoneSurvival 25 ± 2 Three times $n=5$ $(1,1)$ 20Three times a week (M, W, F):Survival a 20 ± 2 Once $n=10$ 1Three times a week (M, W, F):Survival a 20 ± 2 Once $n=10$ 1Three times a week (M, W, F):Survival a 20 ± 2 0 nce $n=10$ 1Twice a week:Survival a 20 ± 2 0 nce $n=10$ 1Twice a week:Survival a 20 ± 2 0 nce $n=10$ 1Twice a week:Survival a 20 ± 2 0 nce $n=5$ 5 $0 ng Tetramarin *beakerSurvivala20 \pm 20 \text{ ng Tetramarin *beaker0 \text{ nother tage:SurvivalSurvival$	Test Method	Temperature (°C)	Water Renewal	Replicates (Chamber Size)	Organisms per Replicate	Feeding Regime	Endpoint(s)	Control Acceptability Criteria	
20 ± 2 None $n=5$ $(1L)$ 20 NoneSurvival 25 ± 2 None $n=5$ $(1L)$ 20 NoneSurvival 25 ± 2 None $n=5$ $n=k$ 20 NoneSurvival 25 ± 2 Three times $n=5$ $n=k$ 20 NoneSurvival 25 ± 2 Three times $n=5$ $n=k$ 20 NoneSurvival 20 ± 2 Once $n=5$ $n=kek$ 0 1 Three times a week (M, W, F): Days 0:14:20 mg Tetramin % becakerSurvival 20 ± 2 Once $n=10$ $neek$ 1 1 Tuesdays: 15:28: 40 mg Tetramain % becakerSurvival 20 ± 2 Once every $n=10$ 1 1 Tuesdays: 2 mg Tetramain % becakerSurvival 20 ± 2 Once every $n=5$ 5 40 mg Tetramain % becakerSurvival	10-day Americamysis bahia	20 ± 2	None	n=5 (1L)	20	<i>Artemia</i> nauplii daily	Survival	≥ 90% survival	Α
25 ± 2 None $n=5$ 20 NoneSurvival 25 ± 2 Three times $n=5$ 20 Three times a week (M, W, F):Survival 25 ± 2 Three times $n=5$ 20 Days 0-14: 20 mg Tetramin®/beakerSurvival 25 ± 2 Once $n=10$ 1Twice a week (M, W, F):Survival 20 ± 2 Once $n=10$ 1Twice a week:Reproduction 20 ± 2 Once $n=10$ 1Twice a week:Survival 20 ± 2 Once every $n=5$ 5GrowthSurvival 20 ± 2 Once every $n=5$ 5Hord days: 2 mg Tetramarin®/beakerSurvival 20 ± 2 Once every $n=5$ 540 mg Tetramarin®/beakerSurvival	10-day Ampelisca abdita	20 ± 2	None	n = 5(1 L)	50	None	Survival	≥ 90% survival	CCEP
$25 \pm 2 \qquad \text{Three times} \qquad n = 5 \qquad \text{per week} \qquad (1 \text{ L}) \qquad 20 \qquad \text{Days } 0.14: 20 \text{ mg Tetramin % Peaker} \qquad \text{Growth} \\ \text{per week} \qquad (1 \text{ L}) \qquad 20 \qquad \text{Days } 15-28: 40 \text{ mg Tetramin % Peaker} \qquad \text{Growth} \\ 20 \pm 2 \qquad \text{Once} \qquad n = 10 \qquad 1 \qquad \text{Twice a week:} \qquad \text{Reproduction} \\ 20 \pm 2 \qquad \text{Once} \qquad n = 10 \qquad 1 \qquad \text{Twice a week:} \qquad \text{Twice a week:} \\ 20 \pm 2 \qquad \text{Once every} \qquad n = 5 \qquad 5 \qquad \text{Hom g Tetramarin % Peaker} \qquad \text{Survival} \\ \text{Growth} \qquad \text{Growth} \\ \text{Ing alfalfa/beaker} \qquad \text{Survival} \qquad \text{Growth} \\ \text{Growth} \qquad \text{Ing alfalfa/beaker} \qquad \text{Survival} \qquad \text{Growth} \\ \text{Once every} \qquad n = 5 \qquad 5 \qquad \text{Hom g Tetramarin % Peaker} \qquad \text{Survival} \qquad \text{Growth} \\ \text{Growth} \qquad \text{Twice days} \qquad (11) \qquad 5 \qquad \text{Hom g Tetramarin % Peaker} \qquad \text{Growth} \qquad \text{Growth} \\ \text{Growth} \qquad \text{Growth} \qquad \text{Growth} \qquad \text{Growth} \qquad \text{True days:} \text{Twice days} \qquad \text{True days} \qquad \text{Survival} \qquad \text{Growth} \qquad \text{True days} \qquad True da$	10-day Leptocheirus plumulosus	25 ± 2	None	n = 5 (1 L)	50	None	Survival	≥ 90% survival	TED
$20 \pm 2 \qquad \text{Once} \qquad n = 10 \qquad 1 \qquad \text{Twice a week:} \\ a \text{ week} \qquad (0.3 \text{ L}) \qquad 1 \qquad \text{Tuesdays: 2 mg Tetramarin}^{\bullet}/\text{beaker} \qquad \text{Survival} \\ \text{Tridays: 2 mg Tetramarin}^{\bullet} \qquad \text{Growth} \\ 1 \text{ mg alfalfa/beaker} \qquad 1 \text{ mg alfalfa/beaker} \qquad \text{Survival} \\ 20 \pm 2 \qquad \text{Once every} \qquad n = 5 \qquad 5 \qquad \text{Every other day:} \qquad \text{Survival} \\ \text{Growth} \end{cases}$	28-day Leptocheirus plumulosus	25 ± 2	Three times per week	n = 5 (1 L)	20	Three times a week (M, W, F): Days 0-14: 20 mg Tetramin [®] /beaker Days 15-28: 40 mg Tetramin [®] /beaker	Survival Growth Reproduction	Survival ≥ 80%, measurable growth Neonates in all renlicates	MANUS
20 ± 2 Once every $n = 5$ 5 Every other day: Survival three days (1 L) 5 $40 \text{ mg Tetramarin}^{\circ}/\text{beaker}$ Growth	28-day Neanthes arenaceodentata	20 ± 2	Once a week	n = 10 (0.3 L)	1	Twice a week: Tuesdays: 2 mg Tetramarin [®] /beaker Fridays: 2 mg Tetramarin [®] 1 mg alfalfa/beaker	Survival Growth	≥ 80% Survival, measurable growth	
	20-day Neanthes arenaceodentata	20 ± 2	Once every three days	n = 5 (1 L)	Ś	Every other day: 40 mg Tetramarin [®] /beaker	Survival Growth	≥ 80% Survival, measurable growth	
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Table 2. Summary of physical characteristics and major classes of organic compounds detected in sediments ($\mu g/kg$) used in the preliminary evaluation (PE) and interlaboratory evaluation (IE). TOC = total organic carbon, PAH = polycyclic aromatic hydrocarbon,	2,3,7,8 TCDD (µg/kg)	
sediments (μg/k = polycyclic aro	ΣDDT (µg/kg)	nzodioxin.
Table 2. Summary of physical characteristics and major classes of organic compounds detected in sediments ($\mu g/kg$) used in the preliminary evaluation (PE) and interlaboratory evaluation (IE). TOC = total organic carbon, PAH = polycyclic aromatic hydroca	Σ PCBs (µg/kg)	PCB = polychlorinated biphenyl, DDT = dichlorodiphenyltrichloroethane, TCDD = tetrachlorodibenzodioxin.
es of organic comp.). TOC = total orga	Σ PAH † (µg/kg)	loroethane, TCDD
and major classe y evaluation (IE)	Bulk Porewater Ammonia (mg/L)‡	orodiphenyltrich
characteristics l interlaborator	TOC (%)	l, DDT = dichl
nary of physical Iuation (PE) and	Particle Size (%)	orinated bipheny
Table 2. Sumr preliminary eva	Sediment	PCB = polychle

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	i.	All		ļ		Ę	Ĺ	Ē		Ę		Ę		Ę
	Fines	Sand	ΡE	ΙĘ	ΥE	ΠĒ	ΡĒ	ΠĒ	ΥE	ΠĘ	ΥE	ΠĒ	ΡE	ΙĒ
Control	71	29	0.6		2.5		< 600		< 30		\sim		n.a.	
Reference	7	98	<0.04	n.a.	n.a.	n.a.	1.6	n.a.	<0.39	n.a.	$\overline{\lor}$	n.a.	< 0.0004	n.a.
Arthur	66	1	4.5	n.a.	77.6	n.a.	7,811	n.a.	204	n.a.	555	n.a.	0.0085	n.a.
Buttermilk	92	8	3.6	n.a.	60.6	n.a.	10,405	n.a.	195	n.a.	15	n.a.	0.0020	n.a.
Chester	40	09	2.7	2.0	36.3	31.4	14,802	13,093	122	162	21	53	0.0006	< 0.0004
Flushing	76	ŝ	5.2	n.a.	57.1	n.a.	14,173	n.a.	271	n.a.	40	n.a.	0.0025	n.a.
Hudson	93	٢	3.4	2.9	47.3	41.4	7,382	5,090	675	720	62	147	0.0180	0.0420
Jamaica Bay	74	27	3.8	n.a.	62.4	n.a.	1,530	n.a.	51	n.a.	pu	n.a.	0.0012	n.a.
100% Newark	92	6	3.2	3.3	32.6	34.4	15,206	8,000	238	251	78	120	0.0280	0.0068
75% Newark	n.a.	n.a.	n.a.	2.6	n.a.	31.3	n.a.	6,869	n.a.	208	n.a.	95	n.a.	0.0041
50% Newark	n.a.	n.a.	n.a.	2.6	n.a.	30.1	n.a.	3,354	n.a.	151	n.a.	49	n.a.	0.0018
25% Newark	n.a.	n.a.	n.a.	2.5	n.a.	26.7	n.a.	1,825	n.a.	82	n.a.	32	n.a.	0.0005
Perth	93	8	3.5	n.a.	19.0	n.a.	2,984	n.a.	112	n.a.	31	n.a.	0.0021	n.a.
Red Hook	46	54	1.7	1.5	35.1	34.8	5,166	94	94	101	31	31	0.0009	0.0011
n.a. = not available	ble													

n.a. = not available
13 PAHs from (Swartz, 1999)
All sediments purged to 20 mg/L total ammonia prior to the addition of test organisms.

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	10-d Acute	ute Tests Mean Survival	Survival	28	28-d Leptocheirus	irus	28-d A	28-d Neanthes
					plumulosus	S	arenacı	arenaceodentata
Sediment	Americamysis bahia (%)	Ampelisca abdita (%)	Leptocheirus plumulosus (%)	Mean Survival (%)	Biomass (mg)	Neonates/ Survivor	Mean Survival (%)	Biomass (mg)
Control	90 ± 9	87 ± 10 †	91 ± 2	9 ∓ 96	0.9 ± 0.5	2.4 ± 1.5	80 ± 42	1.2 ± 0.5
Reference	86 ± 7	$42 \pm 6^*$	72 ± 15	$60 \pm 15^{*}$	0.4 ± 0.0	$0.2 \pm 0.2*$	80 ± 42	1.1 ± 0.5
Arthur	93 ± 8	58 ± 15	$29 \pm 12*$	$71 \pm 17^{*}$	0.9 ± 0.1	0.6 ± 0.5	70 ± 48	0.8 ± 0.4
Buttermilk	78 ± 30	$45 \pm 18^*$	$53 \pm 12^*$	90 ± 10	1.2 ± 0.1	1.7 ± 1.9	80 ± 42	$2.6 \pm 0.9 \#$
Chester	95 ± 5	$55 \pm 23*$	$40 \pm 25^{*}$	$65 \pm 15^*$	0.5 ± 0.1	$0.2 \pm 0.2^{*}$	60 ± 52	$2.7 \pm 0.9 \#$
Flushing	88 ± 3	$42 \pm 19^*$	$37 \pm 27*$	79 ± 13	0.8 ± 0.2	$0.1 \pm 0.2^{*}$	90 ± 32	$2.2 \pm 0.8 \#$
Hudson	90 ± 8	$48 \pm 10^*$	11 ± 4*	$46 \pm 13^{*}$	0.6 ± 0.2	1.9 ± 2.6	70 ± 48	2.1 ± 0.7
Jamaica Bay	94 ± 8	59 ± 28	79 ± 18	89 ± 14	1.3 ± 0.4	0.8 ± 0.4	70 ± 48	1.5 ± 0.9
Newark	82 ± 10	$36 \pm 11^*$	$27 \pm 14^{*}$	$71 \pm 11^{*}$	0.6 ± 0.3	$0.2 \pm 0.4^{*}$	70 ± 48	1.7 ± 0.5
Perth	75 ± 26	$46 \pm 23^{*}$	72 ± 11	84 ± 12	1.0 ± 0.3	1.1 ± 1.0	100 ± 0	1.5 ± 0.4
Red Hook	97 ± 3	72 ± 10	$56 \pm 15^{*}$	89 ± 10	1.0 ± 0.2	0.8 ± 0.8	100 ± 0	$2.5 \pm 0.7 \#$

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- * Statistically significant reduction compared to the control and a response greater than the toxicological decision criterion (i.e., > 20% reduction relative to the control).
 - # Statistically significant increase in response relative to the control.

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