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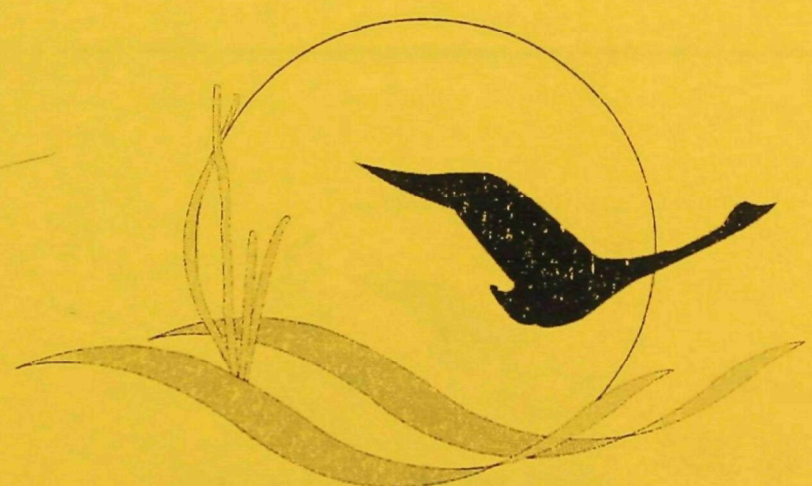
Application of the 10-d Acute and 28-d Chronic *Leptocheirus plumulosus* Sediment Toxicity Tests to the Ambient Toxicity Assessment Program



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FINAL REPORT

Application of the 10-d Acute and 28-d Chronic *Leptocheirus plumulosus* Sediment Toxicity Tests to the Ambient Toxicity Assessment Program

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FOREWORD

This study was designed to compare the current suite of sediment toxicity test methods used by the Ambient Toxicity Assessment Program with the 10-d acute and 28-d chronic *Leptocheirus plumulosus* test method proposed by the U.S. Environmental Protection Agency's (U.S. EPA) Office of Science and Technology. A team of scientists from two Chesapeake Bay research laboratories worked jointly to complete this goal. Mr. Lenwood Hall from the University of Maryland and Dr. Joe Winfield from Old Dominion University played a critical role in technical assistance and project coordination. The sediment toxicity tests using the U.S. EPA's methods were conducted by Dr. Daniel Fisher's research group of the University of Maryland's Wye Research and Education Center. Sediment collection and the routine Ambient Toxicity Assessment Program's sediment toxicity tests were managed by Alan Messing of Old Dominion University's Applied Marine Research Laboratory. The U. S. Environmental Protection Agency's Chesapeake Bay Program Office supported this study.

ABSTRACT

The goal of this report is to compare the U.S. EPA approved *Leptocheirus plumulosus* 10-d acute and 28-d chronic sediment test methods with sediment toxicity test methods used in the Ambient Toxicity Assessment Program. The toxicity of estuarine and freshwater sediments was evaluated during the late summer/early fall of 1998 at four stations in the Choptank River and six stations in the Anacostia River. Sediment samples from these stations were collected and split between two laboratories. The Ambient Toxicity Assessment Program methods included the 10-d sheepshead minnow *Cyprinodon variegatus* embryo/larval test and 20-d survival and growth tests (with 10-d survival data) with the estuarine amphipods *L. plumulosus* and *Lepidactylus dytiscus*, the freshwater amphipod *Hyalella azteca,* and the polychaete worm *Streblospio benedicti.* These tests were conducted by Old Dominion University's Applied Marine Research Laboratory (AMRL). The U.S. EPA *L. plumulosus* tests were conducted by the University of Maryland's Wye Research and Education Center (WREC). The 10-d static acute test used mortality as the endpoint while the 28-d static-renewal exposure used mortality, growth, and reproduction as endpoints.

Results from the WREC testing indicate that the toxicity of the sediments to *L. plumulosus* from the Anacostia River was much greater than that from the Choptank River. *L. plumulosus* survival was not effected in the Choptank River sediments in the acute test but was significantly reduced in four of the six stations in the Anacostia River. The 28-d chronic toxicity test was more sensitive than the 10-d test with survival significantly reduced at all of the Anacostia River stations and at one Choptank River station. In addition, growth rate and/or reproduction was reduced in the three middle Anacostia River stations. The growth rate and reproduction endpoints did not detect any more toxic stations in the 28-d test than the survival endpoint although they did indicate the severity of the toxicity at the three middle river stations. The acute and chronic toxicity followed a gradient down river in the Anacostia River with the most toxic stations occurring in the middle river sections.

The 10-d and 28-d U.S. EPA recommended *L. plumulosus* test methods produced results consistent with the 10-d and 20-d *L. plumulosus* methods used in the Ambient Toxicity Assessment Program. The AMRL 10-d test showed one additional toxic station in the Anacostia River than the WREC test but both tests indicated that AR4 was the most toxic station tested. Neither 10-d test indicated toxicity in the Choptank River. Both chronic methods showed that all stations in the Anacostia were toxic and that a gradient of toxicity existed with the middle river stations most toxic. Both the WREC 28-d test and the AMRL 20-d test showed toxicity at one station in the Choptank River. The AMRL test found slight toxicity at two other Choptank River stations although survival at these stations was reduced by only about 15% from control survival.

Test results were similar for the *L. plumulosus* whole sediment tests conducted by WREC and AMRL and the *H. azteca* tests conducted by AMRL as all chronic tests indicated all stations in the Anacostia to be toxic. In contrast to the ranking from the *L. plumulosus* tests, the *H. azteca* results showed the two upstream stations to be most toxic. This difference in ranking may be a function of the salinity adjustments necessary to conduct the estuarine organism tests using freshwater sediments. Neither the 10-d or 20-d *S. benedicti* or the 10-d *C. variegatus* sediment tests were as sensitive as the *L. plumulosus* or the *H. azteca* tests. In contrast, the *L. dytiscus* test detected toxicity at every station tested based on survival and found the greatest toxicity in the Choptank

River. This species appears overly sensitive to muddy sediments and has been dropped from the Ambient Toxicity Assessment Program.

In conclusion, it appears that the *L. plumulosus* sediment tests conducted by the U.S. EPA method and the Ambient Toxicity Assessment Program method give a level of sensitivity necessary for use as a biomonitoring tool. In addition, both test methods give comparable results. Although there is some question on the effects of salinity adjustment on toxicity in this species, the test was still able to detect all of the stations found to be toxic to the freshwater species *H. azteca*. There appears to be some difference in the ranking of stations when these two species are compared. When used in a multi organism/multi variant data analysis procedure (Hall et al., 2000) where toxicity, sediment chemistry, and benthic community structure are taken into account, both *L. plumulosus* test methods would seem to work equally well in detecting toxic stations.

The U.S. EPA feels that its 28-d *L. plumulosus* method will be the standard whole sediment estuarine toxicity test in the future since it not only measures survival and growth but also reproduction. It has undergone a rigorous validation program including round-robin testing with laboratories throughout the country while the Ambient Toxicity Assessment Program method was developed and is used at only one laboratory. The U.S. EPA method is also somewhat less labor intensive because it does not require a test breakdown in the middle of the exposure period. The similarity between the U.S. EPA 28-d method and the Ambient Toxicity Assessment Program 20-d method found in this study is important because it gives confidence in comparing the earlier Ambient Toxicity Assessment Program work with other datasets generated using the U.S. EPA method in Chesapeake Bay and throughout the East Coast of North America.

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INTRODUCTION

In 1989, research was initiated to develop a chronic sediment toxicity test with benthic amphipods indigenous to Chesapeake Bay. This research, sponsored in part by the U.S. EPA Chesapeake Bay Program and the Office of Science and Technology, culminated in the development of a draft method for assessing the chronic toxicity of marine and estuarine sediments using the amphipod *Leptocheirus plumulosus* (DeWitt et al., 1992). This chronic test measures survival, growth and reproduction after a 28-d exposure to whole sediments. Currently, the U.S. EPA is in the process of finalizing protocols for this 28-d test (DeWitt et al., 1996; U.S. EPA, 1998). Few standardized chronic test methods are available for estuarine and marine sediments, hence, it is anticipated that the 28-d chronic test with *L. plumulosus* will have broad applicability in the environmental testing realm. Already, it has been, and will be, applied in several studies of sediment contamination both within and outside of the Chesapeake Bay (Emery et al., 1996; McGee and Fisher, 1999; McGee et al., 1993, Scott et al., 1996).

The *1994 Chesapeake Bay Basinwide Toxics Reduction and Prevention Strategy* directs the Chesapeake Bay Program Signatories to: "Support and conduct the necessary biological and chemical assessments, including ambient toxicity and community structure, of Bay habitats to ensure future characterization of all tidal Bay habitats through the Regions of Concern identification protocol." The current Ambient Toxicity Assessment Program (1990-1999) has generated data critical for defining geographic areas with existing toxics problems and providing new information for important living resources habitats where no, or limited, data existed. The sediment toxicity methods currently used in this program include: 10-d estuarine sheepshead minnow *Cyprinodon variegatus* embryo/larval test; 20-d estuarine amphipod *Leptocheirus plumulosus* survival and growth test (with 10-d survival data); 20-d freshwater amphipod *Hyalella azteca* survival and growth test (with 10-d survival data); 20-d estuarine amphipod *Lepidactylus dytiscus* survival and growth test; and 20-d estuarine polychaete worm, *Streblospio benedicti* survival and growth test (Hall et al., 1994). One weakness of the existing program is that the sediment test methods used in this effort have not been rigorously evaluated or validated. For example, the 20-d *L. plumulosus* survival and growth test method differs significantly from the 28-d chronic test method developed by the U.S. EPA for the same species. While the U.S. EPA method has undergone a rigorous validation program including round-robin testing with laboratories throughout the country, the Ambient Toxicity Assessment Program method was developed and is used at only one laboratory.

The objective of this report is to compare the U.S. EPA approved 10-d acute and 28-d chronic sediment test with *L. plumulosus* with sediment toxicity methods used in the Ambient Toxicity Assessment Program. The sediment tests were conducted in conjunction with the current Ambient Toxicity Assessment Program using split samples. The incorporation of the two tests into the current repertoire of tests will broaden the utility of the existing Chesapeake Bay Ambient Toxicity database in several ways. First, scientist will have the ability to directly compare toxicity data collected in the Ambient Toxicity Program with other studies conducted in Chesapeake Bay and throughout the nation using the same species and test method. Second, it will be advantageous to compare existing methodologies used in the Ambient Program to a U.S. EPA-standardized approach that has undergone rigorous scientific evaluation and field validation. Finally, some evidence suggests that the reproductive endpoint in the 28-d test may be more sensitive than the survival and growth endpoints currently in use.

MATERIALS AND METHODS

Sample Stations

Ten sampling stations were selected for the 1998 portion of the ongoing Ambient Toxicity Assessment Program. Six stations were located in the Anacostia River (AR1-AR6) in Washington, D.C. and four were in the Choptank River (CR59, CR61, CR62, and CR63) on the Eastern Shore of Maryland. The Choptank River was selected for ambient toxicity testing because it is an ecologically important eastern shore river that has not been tested previously in the ambient toxicity program. Stations selected for testing were located in areas that were also tested by NOAA in their Ambient monitoring program in Chesapeake Bay in 1998. The Anacostia River was selected for ambient toxicity testing because this is the only "Region of Concern" identified by the Chesapeake Bay's Toxic Subcommittee where ambient toxicity data collected from the Ambient Toxicity Assessment Program are lacking. Station depth varied from eight to fifteen feet in the Anacostia and sixteen to twenty-five feet in the Choptank. The Anacostia overlying water was fresh while the Choptank had a salinity of approximately 10‰. The coordinates of the stations are as follows: AR1 (N38 55.159 x W76 56.460), AR2 (N38 54.856 x W76 57.213), AR3 (N38 52.478 x W76 58.999), AR4 (N38 52.263 x W77 00.353), AR5 (N38 51.775 x W77 00.458), AR6 (N38 51.402 x W77 01.276), CR59 (N38 43.879 x W76 15.050), CR61 (N38 41.262 x W76 16.713), CR62 (N38 39.785 x W76 13.845), and CR63 (N38 35.889 x W76 07.515).

Sample Collection, Handling, and Storage

General sediment collection, handling, and storage procedures described in Hall et al. (1991) were used in this study. Samples were collected at each station by Old Dominion University's Applied Marine Research Laboratory (AMRL) personnel and returned to the laboratory for testing. Sediments were collected September 23-24, 1998, by petite ponar grab. The top two centimeters from each grab was used for toxicity testing. Enough sediment was collected at each field replicate site at each station location to supply both AMRL and the WREC for the various sediment toxicity tests. True field replicates were maintained separately and transported to both laboratories. Sediment was collected at each station by first randomly identifying 5 grab sample sites within a 100 meter square grid. At each sample site a discrete field replicate was collected for bioassays and stored on ice. All samples were transported on ice in coolers, out of direct sunlight. Bioassay samples were held in refrigerators at 4°C until initiation of the toxicity tests.

Sediment Toxicity Tests

The 10-d static acute test with *L. plumulosus* used the current U.S. EPA method (U.S. EPA, 1994) with mortality as the endpoint. The chronic sediment toxicity test with this species consisted of a 28-d static-renewal exposure with mortality, growth and reproduction as the endpoints. The method followed the most recent U.S. EPA chronic draft method (U.S. EPA, 1998). Summaries for each test method are contained in Tables 1 and 2. The acute and chronic tests were conducted September 30 - October 9, 1998 and September 29 - October 27, 1998, respectively. Test organisms were obtained from existing cultures at the WREC laboratory by size sorting amphipods through a 600 μm onto a 250 μm sieve for the chronic test and 710 μm onto a 500 μm sieve for the acute test.

Test sediments were press-sieved through a stainless steel 250 μm mesh prior to testing to remove live organisms, particularly indigenous *L. plumulosus* (U.S. EPA/U.S. ACE, 1994) and facilitate recovery of neonates (the reproductive endpoint) at the end of the chronic test. Existing ambient sediment test protocols also require sieving of sediments prior to testing, but only to 500 μm (Hall et al., 1991). Control sediments, collected from a site in the Magothy River, were also sieved to 250 μm. These silty-clay sediments are used to culture the amphipods. Overlying water was Wye River water, filtered to 1 μm and adjusted as necessary with Hawaiian Marine Mix® to 17 to 20‰.

A 96-h water-only reference toxicity test using aqueous cadmium was performed on the same batch of organisms used in the sediment tests. This is the reference test method used in thelatest U.S. EPA draft methods and WREC laboratory Standard Operating Procedure (DeWitt et al., 1996; McGee and Fisher, 1998). A summary of the method is presented in Table 3. Test conditions were those used routinely by the WREC for reference toxicity tests with this species. The trimmed Spearman-Karber method was be used to calculate the median lethal concentration (LC50). The LC50 of an acceptable test must fall within the 2 standard deviation range for the control chart generated at the WREC laboratory.

The sediment toxicity methods currently used in the Ambient Toxicity Assessment Program include: 10-d estuarine sheepshead minnow *Cyprinodon variegatus* embryo/larval test; 20-d estuarine amphipod *Leptocheirus plumulosus* survival and growth test (with 10-d survival data); 20-d freshwater amphipod *Hyalella azteca* survival and growth test (with 10-d survival data); 20-d estuarine amphipod *Lepidactylus dytiscus* survival and growth test (with 10-d survival data); and 20-d estuarine polychaete worm *Streblospio benedicti* survival and growth test (with 10-d survival data). Culture, maintenance, and test procedures used for *S. benedicti* and *L. dytiscus* are described in Hall et al. (1991); *H. azteca* are described in Hall et al. (1992); *Cyprinodon variegatus* eggs and *L. plumulosus* are described in Hall et al. (1994). These tests were conducted by Old Dominion University's Applied Marine Research Laboratory (AMRL) on samples split with the WREC.

Data Analysis

Statistical procedures for the analysis of sediment toxicity test data are presented in U.S. EPA (1994) and U.S. EPA/ACE (1994). The data were analyzed using the statistical package SigmaStat® 2.03 by SPSS, Inc. Data were assessed for normality and homogeneity of variance using the Kolmogorov-Smirnov test and Levene's Median test, respectively. Survival data were Arc Sine Square Root transformed prior to analysis. All data met the normality and homogeneity of variance assumptions. Data were then analyzed via ANOVA followed by comparisons between test sediments and the control using Fisher's LSD test. The 10-d and 28-d *L. plumulosus* test endpoints were compared with other sediment bioassay endpoints obtained by AMRL by assessing the relative agreement in the toxicity ranking of the sediments.

RESULTS AND DISCUSSION

Water Quality

Measurements for water quality during the acute test are given in Table 4 while those for the chronic tests are given in Table 5. Overlying ammonia was low in all test beakers, with the highest

recorded values of 6.0 mg/L from Stations AR2 and AR4 in the acute test and 8.0 mg/L from Station AR4 in the chronic test. These values are well below the level of 60 mg/L that would be considered to be a problem by the U.S. EPA (U.S. EPA, 1998). Values for pH, salinity and dissolved oxygen were within acceptable U.S. EPA ranges (U.S. EPA, 1998).

Reference Toxicant Test

The cadmium chloride reference toxicity test run in conjunction with these sediment tests resulted in a 96-h LC50 of 0.37 mg/L as cadmium. This value falls within the acceptable range (± 2 standard deviations) for cadmium reference toxicity tests conducted at the WREC laboratory (0.28 to 0.39 mg/L as cadmium).

Leptocheirus plumulosus Sediment Toxicity Test Results

The toxicity of the sediments from the Anacostia River was much greater than that from the Choptank River (Table 6). *L. plumulosus* survival in all of the Choptank River sediments in the acute test was greater than 96.0%. Control amphipod survival was 98.0%. In contrast, amphipod survival in sediments from four of the six stations in the Anacostia during the acute test was significantly reduced (AR1, AR3, AR4, and AR5) with the greatest reduction in survival occurring at Station AR4 (6.5% survival). Stations AR1 and AR3 showed only minimal reductions in survival. Actual individual replicate data for both the acute and chronic tests at each station can be found in Table 7 (Anacostia) and Table 8 (Choptank).

L. plumulosus survival was significantly reduced in the chronic test at all of the Anacostia River stations and in Station CR63 in the Choptank River (Table 6). The worst stations appeared to be AR3, AR4, and AR5, with AR4 being the worst overall with only 11.0 % survival. The reduction in survival at the other Anacostia stations and at Station CR63 in the Choptank was not as dramatic as that in AR3 and AR4.

Control amphipod survival met the performance quality assurance criteria for a valid test in the acute test (>90%) and the chronic test (>80%) with the exception of one replicate of the chronic test. There was a serious problem in this replicate. At the midpoint in the test this treatment developed a thick black mat covering the entire surface and all indications of amphipod activity/burrows disappeared. No amphipods were alive at the end of the 28-d test period. Since the other control replicates had high survival and were relatively consistent, it was felt that this treatment was an anomaly and it was not included in the statistical analyses. Since this study, our laboratory has conducted experiments which show that light intensity must be kept at between 300 to 500 lux directly over the entire experimental unit in order to avoid this problem. This appears to have solved the problem with the occasional formation of these thick mats which are believed to be formed by a fungus. No problems have been experienced in ten chronic tests since the light intensity was increased. Previously, this light intensity had been recommended by the U.S. EPA but in the final draft of the method the light intensity will be emphasized and required.

The control treatment for the chronic tests met the performance quality assurance criteria of measurable growth and reproduction for the sublethal endpoints. Reproduction was low although it was measurable. A very small amphipod was used to start this chronic test (250 to 350 μm and 0.036 mg dry weight). It is possible that there was not enough time for an organism of this size to achieve an optimum reproductive state. In recent tests, larger amphipods (400 to 500 μm) have been

used to start the test and neonate production in surviving control organisms has been in the 3.0 to 5.0 neonates/organism range.

Analysis of the sublethal endpoints in the chronic test showed a significant reduction in growth rate at Stations AR4 and AR5 of the Anacostia River but no reduction in growth rate at any of the Choptank River stations (Table 6). The number of neonates per survivor was reduced at Stations AR3, AR4, and AR5 in the Anacostia River but there was no reduction in neonates per survivor at any of the Choptank River stations (Table 6).

Leptocheirus plumulosus Acute vs. Chronic Toxicity

The 10-d acute toxicity test with this amphipod showed four of the six Anacostia River stations to be toxic but none of the Choptank River stations. The greatest toxicity was found at stations AR4 and AR5 with only minor toxicity at Stations AR1 and AR3. The 28-d chronic toxicity test was more sensitive, indicating toxicity at all of the Anacostia River stations and at Station CR63 on the Choptank River. The additional endpoints of growth and reproduction did not detect any more toxic stations in the 28-d test than the survival endpoint although these endpoints did indicate the severity of the toxicity at stations AR3, AR4, and AR5.

Station Ranks according to Leptocheirus plumulosus Sediment Toxicity

The toxicity data allows the stations to be ranked according to severity of toxicity. The toxicity follows a gradient down river in the Anacostia River. The most toxic stations occurred in the mid river sections with most of the endpoints showing reductions from control values at Stations AR3, AR4, and AR5. Survival, growth and reproduction were severely curtailed at AR4 and AR5 and survival and reproduction were reduced at AR3. Only survival was reduced at Stations AR1, AR2 and AR6 and toxicity seemed to be decreasing at Station AR6 where the Anacostia River meets the Potomac River. Since only Station CR63 caused toxicity in the Choptank River a ranking gradient could not be established for this river system.

Comparison of U.S. EPA Leptocheirus plumulosus Test Results with Ambient Toxicity Test Program Leptocheirus plumulosus Test Results

The 10-d and 28-d U.S. EPA recommended *L. plumulosus* test methods conducted by the WREC produced results consistent with the 10-d and 20-d *L. plumulosus* methods used by ODU-AMRL in the Ambient Toxicity Assessment Program (Table 9). These data were provided by AMRL for use in these comparisons and will be published in their entirety in Hall et al. (2000). The AMRL 10-d test showed one additional toxic station in the Anacostia River than the WREC test (AR2). Both tests indicated that AR4 was the most toxic station tested. Neither 10-d test indicated toxicity at any of the Choptank River stations.

Both chronic test methods showed that all stations in the Anacostia were toxic to *L. plumulosus*. The AMRL 20-d test indicated toxic effects on survival but not growth at these stations. Stations AR3 and AR4 were found to be most toxic. The WREC 28-d tests showed the most toxic stations to be AR3, AR4, and AR5 but this test showed severity of toxicity by effects on both growth and reproduction at Stations AR4 and AR5 and reproduction at Station AR3. Thus, both test methods delineated a gradient with the most severe toxicity occurring in the middle portion of the

river. The U.S. EPA method was able to detect differences in the sublethal endpoints of growth and reproduction at these severely toxic stations although these endpoints were not able to detect any additional toxic stations since all were detected using the long term chronic survival endpoint.

Both the WREC U.S. EPA 28-d test method and the AMRL 20-d test method showed toxicity at Station CR63 in the Choptank River. The WREC method indicated toxic effects on survival but not on the sublethal endpoints while the AMRL data showed toxic effects on growth (length) but not survival. The AMRL method indicated toxicity at CR59 and CR62 that the WREC method did not detect. These effects were on survival, not growth. Their statistical procedure was able to detect a 16.2% reduction from control survival at Station CR 59 and a 13.2% reduction at Station CR62 as significantly different. It is uncertain what the ecological relevance of such small reductions in *L. plumulosus* survival in a long term test would be, although this effect would have to be considered only slightly toxic.

There were a couple of differences in the test designs that may have played a factor in the slight differences seen between the two methods. The major difference in the 10-d test methods is that the amphipods were fed in the AMRL method but not in the U.S. EPA method. Another difference is that the AMRL method requires sieving the test sediments to 500 μm while the U.S. EPA method requires sieving to 250 μm to facilitate neonate collection at the end of the test. This may have had an effect on the chemical composition of the final test sediments but the similar test results indicate otherwise.

In conclusion, the two methods produced comparable toxicity results for these split environmental samples. Rankings of toxicity severity from the two methods were also similar. There are a couple of advantages apparent in the U.S. EPA chronic method. First, the method gives a direct measure of effects on reproduction which, in some cases, may prove a more sensitive indicator of environmental effects and would lend itself to use in population modeling. In addition, the U.S. EPA 28-d method is less labor intensive although it does involve counting neonates and an additional eight days of testing. In the 20-d AMRL method there are two separate test breakdowns after day 10 and day 20. Test breakdown is a labor intensive part of any toxicity test. At the 10-d breakdown the amphipods have to be sieved, counted, and transferred back into the test sediment for an additional 10-days. Extra amphipod handling and manipulation of the sediment during this 10-d breakdown could also cause problems with this test.

Comparison of U.S. EPA Leptocheirus plumulosus Test Results with Other Ambient Toxicity Test Program Sediment Test Results

Tests results were similar for the *L. plumulosus* whole sediment tests conducted by WREC and AMRL and the *Hyalella azteca* tests conducted by AMRL (Table 10). These data were provided by AMRL for use in these comparisons and will be published in their entirety in Hall et al. (2000). The *H. azteca* test indicated all stations in the Anacostia River to be toxic both in the 10-d and 20-d test using survival as the endpoint and in the 20-d test using growth as the endpoint. Thus, the chronic tests for both species yielded similar results. The ranking of toxicity is somewhat different though. The *H. azteca* results showed Stations AR1 and AR2 were most toxic followed by AR5, AR3, AR4, and AR6. The down river gradient seen in the *L. plumulosus* tests was not seen here, although toxicity at the mid stations was still significant based on survival and growth. This difference in ranking may be a function of the salinity adjustment necessary for the freshwater sediments of the Anacostia in order to test *L. plumulosus* and the other estuarine species. Salinity

differences have been shown to have an effect on toxicity, especially with regards to metals (Hall and Anderson, 1995). The major similarity between the test methods is that all stations were detected as toxic by both the *L. plumulosus* and the *H. azteca* test methods.

The toxicity results from the other AMRL test species are not as similar as the *L. plumulosus* and *H. azteca* results (Table 10). These data were provided by AMRL for use in these comparisons and will be published in their entirety in Hall et al. (2000). Neither the 10-d or 20-d *Streblospio benedicti* or the 10-d *Cyprinodon variegatus* sediment tests were as sensitive as the *L. plumulosus* or the *H. azteca* tests. The *S. benedicti* 20-d test indicated that Stations AR4 and AR5 were toxic as did these other two tests but this test only showed one other station as toxic (AR2). There were no growth effects with this species. The *C. variegatus* test did not show any of the Anacostia stations as toxic but did pick up slight toxic hits at CR62 and CR63. This fish test was much less sensitive to the sediments from the Anacostia River than the invertebrate species. Past performance of this species in the Ambient Toxicity Assessment Program has shown it to be moderately sensitive in other river systems so its insensitivity to the Anacostia sediments was surprising. The *Lepidactylus dytiscus* test had the opposite problem. It detected toxicity at every station tested based on survival. No effects were found on growth. It did show severe toxicity at Stations AR4 and AR5 in the Anacostia River similar to the *L. plumulosus* and the *H. azteca* tests but it also showed severe toxic effects at CR59 (9% survival) and CR63 (5% survival)(Hall et al., 2000). In fact, this test indicated that these two stations were more toxic than any of the stations in the Anacostia River. This species appears overly sensitive to muddy sediments. In fact, in the ARML mud reference sediment this species had only 12% survival in the 20-d test. *Lepidactylus dytiscus* has recently been dropped from the suite of organisms used in the Ambient Toxicity Assessment Program.

In conclusion, it appears that the *L. plumulosus* sediment test conducted by either the U.S. EPA method or the Ambient Toxicity Assessment Program method gives a level of sensitivity necessary for use as a biomonitoring tool. Although there is some question on the effects of salinity adjustment on toxicity in this species, the test was still able to detect all of the stations found to be toxic to the freshwater species *H. azteca*. There appears to be some difference in the ranking of stations when toxicity to these two species is compared. When used in a multi organism/multi variant data analysis procedure (Hall et al., 2000) where toxicity, sediment chemistry, and benthic community structure are taken into account, both *L. plumulosus* test methods would seem to work equally well in detecting toxic stations.

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Table 1. Test conditions for 10-d acute sediment toxicity tests with *Leptocheirus plumulosus.*

1. Test type	Whole sediment, static
2. Temperature	25 °C
3. Overlying water	Filtered Wye River water adjusted as necessary with well water or Hawaiian Marine Mix® to 17 to 20‰
4. Light	Ambient laboratory
5. Photoperiod	16:8 (L/D)
6. Test chamber	1 L glass beaker covered with watchglass
7. Sediment volume	175 ml (2 cm)
8. Overlying water volume	800 ml
9. Water renewal	None
10. Size and life stage of amphipods	2-4 mm, sub-adults
11. Number of organisms/replicate	20
12. Number of replicates	5
13. Feeding	None
14. Aeration	1-2 bubbles/sec with 1 ml pipette
15. Water quality	Salinity, pH and total ammonia at beginning and end of test; Temperature and D.O. daily
16. Test duration	10 d
17. Endpoint	Survival
18. Performance criteria	Control survival $\geq$ 90%

Table 2. Test conditions for 28-d chronic sediment toxicity tests with *Leptocheirus plumulosus*.

1. Test type	Whole sediment, static renewal
2. Temperature	25 °C
3. Overlying water	Filtered Wye River water adjusted as necessary with well water or Hawaiian Marine Mix® to 17 to 20‰
4. Light	Ambient laboratory
5. Photoperiod	16:8 (L/D)
6. Test chamber	1 L glass beaker covered with watch glass
7. Sediment volume	175 ml (2 cm)
8. Overlying water volume	800 ml
9. Water renewal	3 x /week, replace 400 ml
10. Size and life stage of amphipods	neonates; size sorted on nested 250 and 500 μm mesh sieves
11. Number of organisms/replicate	20
12. Number of replicates	5
13. Feeding	TetraMin 3x/week
14. Aeration	1-2 bubbles/sec with 1 ml pipette
15. Water quality	Salinity, pH and total ammonia at beginning and end of test; Temperature and D.O. daily
16. Test duration	28 d
17. Endpoints	Survival, growth, reproduction
18. Performance criteria	Control survival $\geq$ 80% Measurable growth and reproduction

Table 3. Test conditions for reference toxicity tests with *Leptocheirus plumulosus*.

1. Test type	Aqueous, static
2. Temperature	25 °C
3. Toxicant	Cadmium
4. Diluent water	Filtered Wye River water adjusted as necessary with well water or Hawaiian Marine Mix® to 8‰.
5. Light	Ambient laboratory
6. Photoperiod	16:8 (L/D)
7. Test chamber	300 ml glass beaker
8. Water volume	200 ml
9. Size and life stage of amphipods	2-4 mm, sub-adults
10. Number of organisms/replicate	10
11. Minimum number of replicates	2
12. Feeding	None
13. Substrate	None
14. Aeration	None
15. Water quality	D.O., salinity, pH, hardness and alkalinity at the beginning and end of test; Temperature daily
16. Duration	96 h
17. Endpoint	Survival
18. Test acceptability	90 % control survival

Table 4. Water quality summary for the 10-d acute *Leptocheirus plumulosus* sediment toxicity test.

Test Type / Date	Temperature (°C)		DO (mg/L)		pH	Salinity (‰)		Ammonia Overlying
Treatment	Mean	SD	Mean	SD	Range	Mean	SD	Range (mg/L)
Acute: 9/30-10/9								
Control	24.5	0.89	6.8	0.28	7.88 - 8.37	17.3	0.55	<0.1 - 0.6
AR 1	24.4	1.11	6.8	0.32	6.91 - 8.44	17.5	0.55	<0.1 - 6.0
AR 2	24.5	1.05	6.7	0.35	6.65 - 8.27	17.0	0.84	<0.1 - 6.2
AR 3	24.5	1.03	6.6	0.47	6.97 - 8.27	17.1	0.45	<0.1 - 4.6
AR 4	24.4	1.04	6.7	0.39	7.51 - 8.04	17.0	0.45	1.7 - 8.0
AR 5	24.4	1.04	6.6	0.56	6.79 - 8.08	17.2	0.55	2.7 - 4.8
AR 6	24.4	0.96	6.8	0.34	7.55 - 7.86	17.0	1.64	3.2 - 5.8
CR 59	24.5	1.03	6.7	0.47	7.84 - 8.44	17.8	1.48	<0.1 - 5.0
CR 61	25.1	0.26	6.7	0.42	7.97 - 8.55	18.3	1.34	<0.1 - 2.1
CR 62	25.1	0.23	6.7	0.49	7.85 - 8.53	18.3	1.41	<0.1 - 6.2
CR 63	25.1	0.30	6.7	0.37	7.66 - 8.51	18.8	0.89	<0.1 - 4.0

Table 5. Water quality summary for the 28-d chronic *Leptocheirus plumulosus* sediment toxicity test.

Test Type / Date	Temperature (°C)		DO (mg/L)		pH	Salinity (‰)		Ammonia Overlying
Treatment	**Mean**	**SD**	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range (mg/L)**
Chronic: 9/29-10/27								
Control	24.7	0.63	6.8	0.38	7.28 - 8.40	18.0	1.78	<0.1 - 0.7
AR 1	24.7	0.54	6.8	0.37	6.89 - 8.14	17.8	1.74	<0.1 - 6.0
AR 2	24.5	0.97	6.6	0.49	7.34 - 8.24	18.0	1.56	<0.1 - 6.0
AR 3	24.7	0.62	6.7	0.33	7.43 - 8.18	18.0	1.72	<0.1 - 3.2
AR 4	24.7	0.60	6.7	0.42	6.63 - 8.29	17.8	1.61	<0.1 - 6.0
AR 5	24.7	0.65	6.6	0.37	7.63 - 8.11	17.8	1.62	<0.1 - 3.0
AR 6	24.6	0.66	6.6	0.38	7.66 - 8.16	18.1	1.73	<0.1 - 5.4
CR 59	24.5	0.82	6.8	0.23	7.21 - 8.10	18.7	2.13	<0.1 - 2.8
CR 61	24.6	0.63	6.6	0.46	7.96 - 8.46	18.8	1.51	<0.1 - 3.0
CR 62	24.6	0.64	6.8	0.37	7.83 - 8.55	18.6	1.43	<0.1 - 3.0
CR 63	24.6	0.61	6.7	0.32	7.61 - 8.22	18.5	1.39	<0.1 - 3.2

Table 6. Summary of the toxicity test results for 10-d and 28-d *Leptocheirus plumulosus* sediment tests conducted at the University of Maryland's Wye Research and Education Center.

	10-d survival (%)		28-d survival (%)		28-d growth rate		28-d neonates per survivor	
Station	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	98.0	2.74	93.8	6.29	0.032	0.0036	0.57	0.184
AR1	75.0*	20.9	65.0*	33.00	0.029	0.0048	0.58	0.064
AR2	87.0	18.9	55.0*	17.00	0.029	0.0034	0.56	0.082
AR3	79.0*	26.6	45.0*	7.07	0.027	0.0030	0.28*	0.192
AR4	6.5*	7.78	11.0*	14.30	0.006*	0.0055	0.03*	0.064
AR5	40.0*	15.40	55.0*	20.30	0.020*	0.0047	0.05*	0.103
AR6	96.0	4.18	57.0*	23.60	0.030	0.0034	0.57	0.156
CR59	96.0	6.52	75.0	31.20	0.034	0.0041	0.59	0.140
CR61	98.0	4.47	93.0	15.70	0.035	0.0049	0.49	0.139
CR62	98.0	2.74	95.0	8.66	0.028	0.0024	0.52	0.147
CR63	97.0	4.47	57.0*	21.10	0.029	0.0005	0.60	0.114

*Significantly less than controls ($p < 0.05$): ANOVA followed by Fisher's LSD

Table 7. Sediment test toxicity data for the Anacostia River sample stations for tests conducted at the University of Maryland's Wye Research and Education Center.

			10-d Acute Test		28-d Chronic Test					
Treatment	Rep	Position[1]	# Alive	% Alive	# Alive	% Alive	Rep wt (mg)	growth rate[2]	# young	young/ survivor
Control	1	8	21	100	17	85	0.904	0.031	14	0.824
	2	7	19	95	19	95	1.080	0.037	11	0.579
	3	1	20	100	19	95	0.885	0.030	8	0.421
	4	13	19	95	20	100	0.839	0.029	9	0.450
	5	24	20	100	0[3]	0	0	0.000	0	0.000
AR1	1	14	20	100	19	95	1.079	0.037	11	0.579
	2	39	19	95	18	90	0.793	0.027	9	0.500
	3	48	12	60	15	75	0.847	0.029	8	0.533
	4	54	13	65	10	50	0.719	0.024	6	0.600
	5	21	11	55	3	15	0.880	0.030	2	0.667
AR2	1	16	20	100	13	65	0.743	0.025	8	0.615
	2	34	11	55	7	35	0.807	0.028	4	0.571
	3	22	17	85	15	75	0.858	0.029	7	0.467
	4	35	19	95	12	60	0.979	0.034	8	0.667
	5	4	20	100	8	40	0.895	0.031	4	0.500
AR3	1	12	20	100	8	40	0.903	0.031	4	0.500
	2	44	20	100	10	50	0.819	0.028	3	0.300
	3	33	10	50	10	50	0.820	0.028	4	0.400
	4	43	19	95	10	50	0.764	0.026	2	0.200
	5	51	10	50	7	35	0.680	0.023	0	0.000
AR4	1	26	0	0	7	35	0.344	0.011	1	0.143
	2	38	1	5	2	10	0.290	0.009	0	0.000
	3	37	1	5	2	10	0.315	0.010	0	0.000
	4	2	4	20	0	0	0	0.000	0	0.000
	5	23	0	0	0	0	0	0.000	0	0.000
AR5	1	6	10	50	11	55	0.596	0.020	0	0.000
	2	15	8	40	4	20	0.455	0.015	0	0.000
	3	46	5	25	13	65	0.652	0.022	0	0.000
	4	50	5	25	14	70	0.512	0.017	0	0.000
	5	31	12	60	13	65	0.791	0.027	3	0.231
AR6	1	25	20	100	18	90	0.772	0.026	7	0.389
	2	11	19	95	7	35	0.930	0.032	4	0.571
	3	9	18	90	7	35	0.960	0.033	3	0.429
	4	3	19	95	14	70	0.906	0.031	10	0.714
	5	19	20	100	11	55	0.758	0.026	8	0.727

[1]Tests started at same time in separate beakers but used identical randomization for position.
[2]Growth rate = (Rep weight - initial weight of 0.036 mg)/28.
[3]Replicate had massive fungal infection that appeared to kill all amphipods. Excluded from statistical analyses.

Table 8. Sediment test toxicity data for the Choptank River sample stations for tests conducted at the University of Maryland's Wye Research and Education Center.

			10-d Acute Test		28-d Chronic Test					
Treatment	Rep	Position[1]	# Alive	% Alive	# Alive	% Alive	Rep wt (mg)	growth rate[2]	# young	young/ survivor
Control	1	8	21	100	17	85	0.904	0.031	14	0.824
	2	7	19	95	19	95	1.080	0.037	11	0.579
	3	1	20	100	19	95	0.885	0.030	8	0.421
	4	13	19	95	20	100	0.839	0.029	9	0.450
	5	24	20	100	0[3]	0	0	0.000	0	0.000
CR59	1	10	20	100	11	55	0.913	0.031	6	0.545
	2	30	20	100	22	100	1.098	0.038	11	0.500
	3	32	17	85	18	90	0.983	0.034	9	0.500
	4	28	19	95	6	30	0.852	0.029	5	0.833
	5	45	20	100	20	100	1.113	0.038	11	0.550
CR61	1	17	18	90	20	100	0.973	0.033	10	0.500
	2	29	20	100	21	100	1.163	0.040	14	0.667
	3	40	24	100	21	100	1.040	0.036	6	0.286
	4	42	20	100	20	100	1.134	0.039	9	0.450
	5	52	20	100	13	65	0.813	0.028	7	0.538
CR62	1	18	20	100	20	100	0.923	0.032	14	0.700
	2	5	20	100	16	80	0.790	0.027	10	0.625
	3	55	20	100	19	95	0.782	0.027	10	0.526
	4	36	19	95	20	100	0.766	0.026	8	0.400
	5	49	19	95	20	100	0.789	0.027	7	0.350
CR63	1	41	20	100	13	65	0.838	0.029	9	0.692
	2	27	18	90	14	70	0.810	0.028	7	0.500
	3	47	20	100	8	40	0.809	0.028	6	0.750
	4	53	20	100	6	30	0.857	0.029	3	0.500
	5	20	19	95	16	80	0.848	0.029	9	0.563

[1]Tests started at same time in separate beakers but used identical randomization for position.
[2]Growth rate = (Rep weight - initial weight of 0.036 mg)/28.
[3]Replicate had massive fungal infection that appeared to kill all amphipods. Excluded from statistical analyses.

Table 9. Comparison of endpoints between *Leptocheirus plumulosus* toxicity tests conducted at the University of Maryland's Wye Research and Education Center (WREC) and Old Dominion University's Applied Marine Research Laboratory (AMRL) on samples from the Anacostia River and the Choptank River.

	Acute Tests		Chronic Tests					
	WREC 10-d	AMRL 10-d	WREC 28-d	AMRL 20-d	WREC 28-d	AMRL 20-d		WREC 28-d
Station	% survival	% survival	% survival	% survival	Growth Rate (mg/d)	Weight (mg)	Length (mm)	Young/survivor
Control	98.0[1]	90.7	93.8	90.7	0.032	0.271	6.387	0.57
AR1	75.0*	66.7*	65.0*	45.3*	0.029	0.262	6.447	0.58
AR2	87.0	56.0*	55.0*	38.7*	0.029	0.278	6.727	0.56
AR3	79.0*	57.3*	45.0*	20.0*	0.027	0.315	6.766	0.28*
AR4	6.5*	32.0*	11.0*	8.0*	0.006*	0.232	6.313	0.03*
AR5	40.0*	68.0*	55.0*	38.7*	0.020*	0.232	6.371	0.05*
AR6	96.0	82.7	57.0*	66.7*	0.030	0.307	6.325	0.57
CR59	96.0	90.7	75.0	76.0*	0.034	0.242	5.968	0.59
CR61	98.0	90.7	93.0	81.3	0.035	0.393	6.305	0.49
CR62	98.0	92.0	95.0	78.7*	0.028	0.251	5.969	0.52
CR63	97.0	89.3	57.0*	82.7	0.029	0.193	5.835*	0.60

[1] Values are the mean for each station.

* Station significantly different from the control station ($p < 0.05$)

Table 10. Comparison of sediment toxicity at each station in the Anacostia and Choptank Rivers versus test method conducted at the University of Maryland's Wye Research and Education Center (WREC) and Old Dominion University's Applied Marine Research Laboratory (AMRL). The number in parentheses equals the station rank in each River System with 1 being the most toxic.

	WREC		AMRL								
	Leptocheirus plumulosus		*Leptocheirus plumulosus*		*Hyalella azteca*[1]		*Lepidactylus dytiscus*		*Streblospio benedicti*		*Cyprinodon variegatus*
Station	10d	28d	10d	28d	10d	20d	10d	20d	10d	20d	10d
AR1	S[2](3)	S (6)	S (4)	S (4)	S (2)	S,G (2)	S (3)	S (4)			
AR2		S (4)	S (2)	S (3)	S (1)	S,G (1)	S (5)	S (4)	S (2)	S (1)	
AR3	S (4)	S,R (3)	S (3)	S (2)	S (4)	S,G (4)	S (1)	S (3)			
AR4	S (1)	S,G,R (1)	S (1)	S (1)	S (3)	S,G (5)	S (5)	S (1)	S (1)	S (1)	
AR5	S (2)	S,G,R (2)	S (5)	S (3)	S (4)	S,G (3)	S (2)	S (2)		S (2)	
AR6		S (5)		S (5)	S (5)	S,G (6)	S (4)	S (3)			
CR59				S (1)			S (2)	S (2)			
CR61								S (4)			
CR62				S (2)			S (3)	S (3)			S,H (1)
CR63		S (1)		G (3)			S (1)	S (1)	S (1)		S,H (2)

[1] *Hyalella azteca* only used to test the freshwater sediments of the Anacostia River.

[2] Significant effects ($p < 0.05$) on the various endpoints tested are indicated by letters (S = survival; G = Growth; R = Reproduction; H = Hatching success).