Lower Passaic River Restoration Project

2012 SEDIMENT TOXICITY REFERENCE DATA FOR THE LOWER PASSAIC RIVER STUDY AREA
FINAL

Prepared for:

Cooperating Parties Group
Newark, NJ

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Acronyms

AFDW	ash-free dry weight
ASTM	American Society for Testing and Materials
AVS/SEM	acid volatile sulfide / simultaneously extracted metal
BERA	baseline ecological risk assessment
COC	chain–of-custody
CPG	Cooperating Parties Group
DGPS	differential global positioning system
DMO	Dinnel Marine Resources
DO	dissolved oxygen
EIS	EnviroSystems, Inc.
GIS	geographic information system
LC50	concentration that is lethal to 50% of an exposed population
LPR	Lower Passaic River
LPRSA	Lower Passaic River Study Area
PMF	Protocol Modification Form
QAPP	quality assurance project plan
QA/QC	quality assurance / quality control
RM	river mile
RI/FS	remedial investigation / feasibility study
SOP	standard operating procedure
SQT	sediment quality triad
TPH	total petroleum hydrocarbons
USEPA	US Environmental Protection Agency

1 Introduction

The Lower Passaic River Study Area (LPRSA, also referred to as the Site) is the 17.4-mile-long stretch of the Passaic River between Dundee Dam and Newark Bay that is the subject of a remedial investigation/feasibility study (RI/FS). It is situated within the Lower Passaic River (LPR) watershed, which is highly urbanized and receives substantial inputs of industrial and municipal discharges. A baseline ecological risk assessment (BERA) will be conducted as part of the RI/FS, and will be used to evaluate the potential for hazardous substances present in environmental media to impact the health of ecological receptors within the LPRSA.

It is important to characterize background concentrations of contaminants in surface water, sediment, and tissue in order to identify the degree to which inputs of chemicals of concern are from sources upstream of the LPRSA. Likewise, it is important to obtain reference information to establish reference conditions for the Site.[1] The evaluation of background chemical concentrations and reference information will be used to assess Site-related risks in context with risks resulting from exposure to regional background (i.e., non-Site-related) sources.

For this reason, Appendix B to the *Revised Risk Analysis and Risk Characterization Plan for the Lower Passaic River Study Area* (Windward and AECOM [in prep]) recommends investigations above Dundee Dam to obtain freshwater reference information for comparison with data collected in the LPRSA. [2]

Reference sediment samples for sediment quality triad (SQT) analysis[3] were collected in November 2012 from the area of the LPR immediately above Dundee Dam. Collection methods followed those presented in the *Lower Passaic River Restoration Project Quality Assurance Project Plan: Surface Sediment Chemical Analyses and Benthic Invertebrate Toxicity and Bioaccumulation Testing* (Windward 2009), hereafter referred to as the Benthic Quality Assurance Project Plan (QAPP), and the *Lower Passaic River Restoration Project Background and Reference Conditions Addendum to the Quality Assurance Project Plan: Surface Sediment Chemical Analyses and Benthic Invertebrate Toxicity and Bioaccumulation Testing*, hereafter referred to as the Benthic QAPP Addendum No. 5 (Windward 2012).

This data report presents the results from the toxicity testing component of the SQT reference sediment samples analysis. The results of the benthic invertebrate community survey and the analysis of chemistry samples will be presented in separate reports (Windward [in prep]).

[1] Appendix B to the *Revised Risk Analysis and Risk Characterization Plan for the Lower Passaic River Study Area* (Windward and AECOM [in prep]) provides a detailed, US Environmental Protection Agency (USEPA)-approved definition of background concentrations and reference information.

[2] Reference datasets are available for comparison to sample data collected in the estuarine portion of the LPRSA.

[3] SQT samples were analyzed for chemistry, toxicity, and benthic invertebrate community indices.

1.1 PURPOSE AND SCOPE

The freshwater sediment toxicity investigation above Dundee Dam was conducted under the authority of the May 2007 Administrative Settlement Agreement and Order on Consent (Section IX.37.d.) (USEPA 2007) between the USEPA and the Cooperating Parties Group (CPG), a consortium of approximately 70 companies that agreed to complete the RI/FS of the 17.4-mile-long stretch of the Passaic River between Newark Bay and Dundee Dam.

The primary objectives of the 2012 sediment collection program were to collect freshwater background sediment chemistry data from one set of locations, and SQT data, which included the collection of additional sediment chemistry data, from another set of locations to establish an upstream reference area. These data will be used to provide context for Site-related risks with regard to the risks resulting from exposure to regional background (i.e., non-Site-related) sources. The sediment toxicity data collected upriver of Dundee Dam as part of the SQT reference dataset will be used to establish a reference condition for the LPRSA sediment toxicity data.

1.2 DOCUMENT OVERVIEW

This document describes the results of the freshwater sediment toxicity reference testing conducted in the 4.1-mile-long stretch of the Passaic River upstream of Dundee Dam. Section 2 presents the sampling design and methodology. Section 3 presents the toxicity test results, followed by a brief summary in Section 4. References are provided in Section 5. The text is supported by the following appendices:

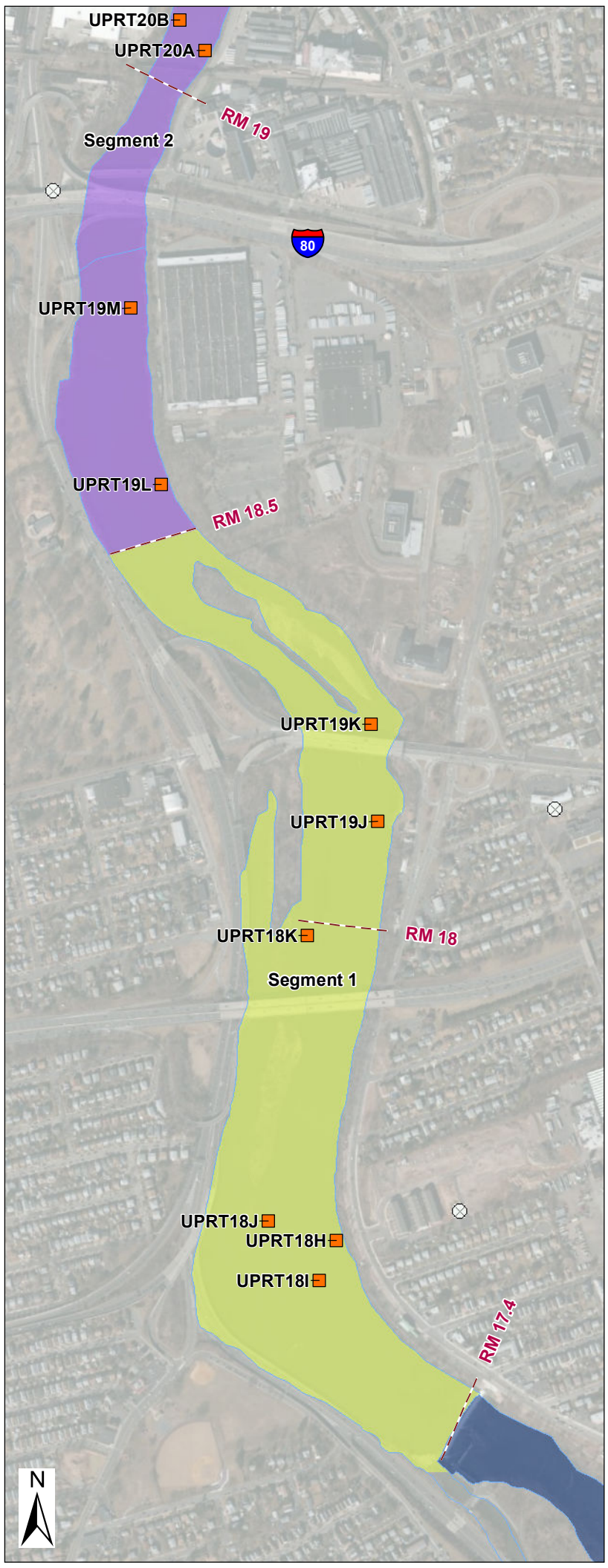
- Appendix A. Sampling Locations
- Appendix B. Field Records
- Appendix C. Data Summary Tables
- Appendix D. Laboratory Reports
- Appendix E. Validation Report
- Appendix F. Chain-of-Custody Forms
- Appendix G. Protocol Modification Forms

2 Sampling Design and Methodology

The sampling design and methodology for the 2012 freshwater reference sediment collection effort above Dundee Dam was presented in the Benthic QAPP Addendum No. 5 (Windward 2012). This section summarizes the elements of the sampling design and methodology that are relevant to the toxicity testing component of the program. Section 2.1 identifies the locations sampled during the 2012 SQT reference sample collection effort conducted above Dundee Dam. Details on the methods used to collect and process surface sediment (0- to 15-cm sediment horizon) samples for toxicity testing are presented in Section 2.2. Section 2.3 presents an overview of the methods used by the toxicity testing laboratory (including quality assurance / quality control [QA / QC] and validation) and the methods used to evaluate the test results.

2.1 SAMPLING LOCATIONS

As specified in the USEPA-approved Benthic QAPP Addendum No. 5 (Windward 2012), freshwater sediment toxicity reference samples were collected at 24 SQT locations between river mile (RM) 17.4 and RM 21.5 (Figure 2-1). The coordinates for each sampling location are provided in Appendix A, Table A-1. The total sampling area was subdivided into four segments: one 1.1-mile segment (the first segment above Dundee Dam from RM 17.4 to RM 18.5) and three 1-mile segments (RM 18.5 to RM 19.5, RM 19.5 to RM 20.5, and RM 20.5 to RM 21.5). Sampling locations were selected in each segment to provide as even a spatial allocation of samples as possible.



SQT location

Benthic SQT

- Segment 1 (RM 17.4 to RM 18.5)
- Segment 2 (RM 18.5 to RM 19.5)
- Segment 3 (RM 19.5 to RM 20.5)
- Segment 4 (RM 20.5 to RM 21.5)

Discharge

- CSO
- River Mile
- River
- LPRSA

0 500 1,000 Feet

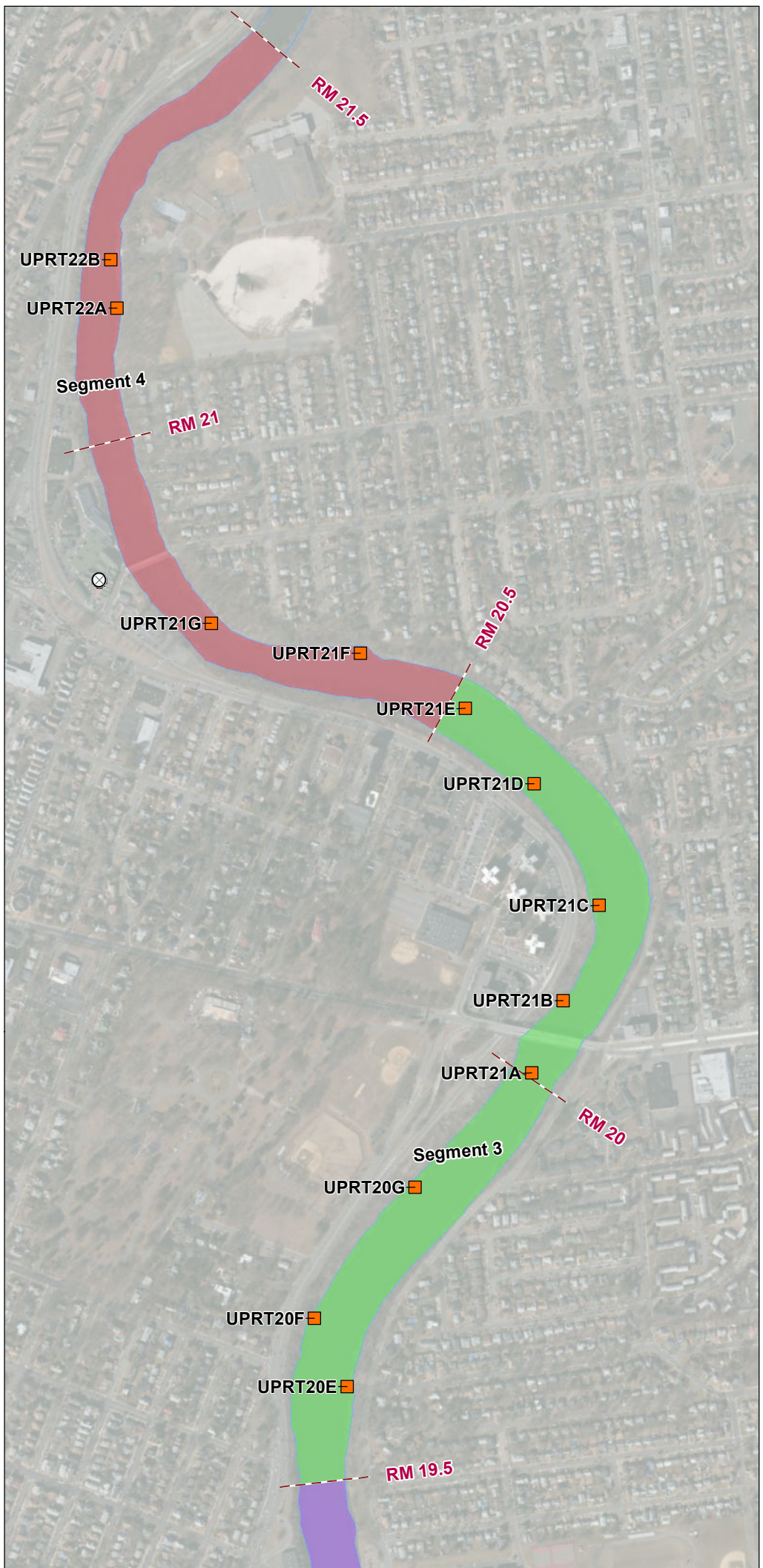


Figure 2.1. Locations where SQT reference samples were collected above Dundee Dam

Wind Ward environmental LLC

Data sources: river miles were derived from USGS NHD Streams.
Imagery from ESRI; http://goto.arcgisonline.com/maps/World_Imagery

Prepared by LSM 8/23/2013; W:\Projects\06-58-01 Passaic RI\Data\GIS\Maps_and_Analysis\Upstream\5417_Upstream actual SQT sed locs_LSM_20121116.mxd

For the SQT data collected within the LPRSA, approximately half of the SQT samples in shallow depth areas were targeted as fine-grained sediment, and approximately half were targeted as coarse-grained sediment.[4] Therefore, to be consistent, 12 of the 24 selected SQT locations above Dundee Dam were targeted as fine-grained samples. Grain size data from previous sampling events above Dundee Dam (i.e., USEPA 2007 sampling (ddms 2011) and CPG 2008 low-resolution core sampling (AECOM [in prep])) facilitated the selection of SQT locations in the first segment (RM 17.4 to RM 18.5). No grain size data were available for the remaining three segments (RM 18.5 to RM 19.5, RM 19.5 to RM 20.5, and RM 20.5 to RM 21.5). Consequently, SQT sampling locations in these three segments were selected based on expected grain size using stream morphology and geographic information system (GIS) data. Expected depositional areas (e.g., areas inside river bends) or areas below bridge abutments were expected to have fine-grained sediment, and potential scouring areas (e.g., areas on the outside of river curves) were assumed to have coarse-grained sediment.

Prior to sediment sampling, a two-day reconnaissance survey was conducted on October 23 and 24, 2012, to verify sampling location accessibility and confirm the grain size at the targeted locations. Grain size confirmation in the field was determined using the wet sieving methods described in Attachment AA of the Benthic QAPP Addendum No. 5 (Windward 2012). Locations that could not be accessed by boat due to shallow water conditions or underwater obstructions (i.e., a utility line crossing the river obstructed access to locations immediately above Dundee Dam) were replaced with new locations; coordinates for the new locations were recorded using a boat-mounted differential global positioning system (DGPS). A protocol modification form (PMF) documenting the changes in locations is provided in Appendix G; see Section 2.2.3 for further discussion.

A USEPA Region 2 contractor authorized to perform oversight duties (i.e., CDM Smith) was present during both the reconnaissance survey and the sediment sampling efforts.

2.2 FIELD SAMPLING METHODS

This section presents the freshwater sediment toxicity reference sample collection, handling, and processing methods that were used during the 2012 freshwater reference sediment collection effort conducted above Dundee Dam. Sediment for chemistry and benthic invertebrate community analyses was collected at the same time as sediment for toxicity testing; the processing of those samples is described in separate reports (Windward [in prep]).

[4] Fine-grained sediment is defined as having ≥ 60% fines (fines are the sum of silt and clay fractions that pass through a No. 200 sieve [i.e., less than 75 µm in diameter]). Coarse-grained sediment is defined as having < 60% fines.

2.2.1 Sample collection

The procedures used to collect and process sediment toxicity samples followed the standard freshwater methods presented in the Benthic QAPP (Windward 2009) and the Benthic QAPP Addendum No. 5 (Windward 2012).

A boat-mounted DGPS system was used to locate the selected sampling locations. Prior to sampling, location coordinates were entered into the DGPS. The actual position was noted using the DGPS once the sampling equipment had been deployed and was positioned on the river bottom. Water depth at each sampling location was measured using a lead line marked in tenths of feet. Water quality parameters (i.e., temperature, dissolved oxygen [DO], pH, and conductivity) were measured at each location using a multi-probe meter that was calibrated daily using standard solutions. Sampling began at the downstream end of the sampling area and proceeded upstream.

Surface (i.e., 0- to 15-cm depth horizon) sediment samples for SQT analysis were collected using a stainless steel pneumatic power grab sampler with a 5-gal. capacity and a 0.2-m^2 surface area. The sampler was deployed from a pontoon boat equipped with a davit and winch. Sampling methods used during the field program are described below, and are also detailed in the standard operating procedure (SOP) included as Attachment D to the Benthic QAPP Addendum No. 5 (Windward 2012).

The number of surface grab samples collected at each location varied depending on the volume required, as well as the substrate and ease of sediment collection. In general, a minimum of five acceptable grab samples (one for chemistry and toxicity testing, and four for benthic invertebrate community analysis [one grab sample for each of the four benthic invertebrate community replicates])[5] were required at each location. An additional grab sample was collected when a field duplicate or USEPA split sample was required, or when there was insufficient sediment available to meet volume requirements for chemistry and toxicity testing. The actual number of grab samples collected at each location is provided in Appendix B (Table B-2), which documents the data collected in the field. The coordinates provided in Appendix B reflect the position of the grab sample collected farthest downstream at each location.

The power grab sampler was deployed from the sampling vessel using a winch to control the speed. Once the power grab sampler had been pulled up and brought on board the boat, it was placed on a stand and evaluated to ensure that the grab was acceptable. A sediment grab was considered acceptable if the sampler had penetrated to a minimum depth of 16 cm (to ensure that sediment could be collected to a depth of 15 cm and had not been in contact with the sampler frame), but had not over penetrated such that the sediment had come into contact with the top of the sampler frame. The total depth of sediment in the grab sampler was determined using a ruler to measure

[5]The methodology and results from the collection of sediment for chemistry and benthic invertebrate community analyses are described in separate reports (Windward [in prep]).

the distance between the surface of the sediment in the grab sample and the top of the 22-cm-deep power grab sampler frame.

Once a grab sample was determined to be acceptable, the overlying water was siphoned and discarded. The subsample of sediment for toxicity testing and chemistry analysis[6] was then transferred to a decontaminated stainless steel container using a decontaminated stainless steel spoon and immediately transferred to a nearby processing boat for homogenization.

Excess sediment from the samples or sediment from unacceptable grab samples was returned to the collection site. If a successful grab sample could not be collected (e.g., as a result of bottom debris or gravel), the sampling location was replaced with an alternative location that was still within the defined target sampling area approved by USEPA (i.e., within 10 m of the proposed sampling location as defined in the Benthic QAPP (Windward 2009)).

2.2.2 Sample handling and processing

Once on the processing boat, the sediment samples collected from each freshwater SQT station reference samples collected for chemistry analysis and toxicity testing were thoroughly homogenized together in order to make one uniform sample.[7] Any large, non-sediment items, such as rocks, shells, wood chips, or large organisms (e.g., clams), were removed prior to homogenization; the surfaces of these items were scraped to remove any sediment and invertebrates, which were homogenized with the rest of the sample. Homogenized sediment was then distributed to the appropriate sample containers for the specific analyses.[8] Sediment for toxicity testing was distributed into 1-gal. Teflon®-lined buckets, tightly sealed, labeled, and stored on wet ice in coolers.

A USEPA Region 2 contractor authorized to perform oversight duties (i.e., CDM Smith) was present during sample handling and processing.

Samples were transported to the CPG field facility at the end of the day, where they were stored at 4 ± 1°C in a walk-in refrigerator. At the end of the SQT reference sediment sampling effort, the samples for toxicity testing were picked up by a courier and delivered to EnviroSystems, Inc. (ESI) in Hampton, New Hampshire.

[6] Note that sediment for the analysis of acid volatile sulfide / simultaneously extracted metals (AVS / SEMs), ammonia, sulfide, and total petroleum hydrocarbons (TPH) purgeables was subsampled directly from the grab sampler immediately after the sampler had been brought on board the sampling vessel. Samples for AVS / SEM, ammonia, sulfide, and TPH purgeables were subsampled as discrete, non-homogenized samples and immediately placed on ice.

[7] Samples collected for benthic invertebrate community analysis were handled and processed separately from samples collected for chemistry analysis and toxicity testing; these methods are described in a separate report (Windward [in prep]).

[8] See the sediment chemistry report (Windward [in prep]) for additional details on the processing of samples for chemistry analysis.

Chain-of-custody (COC) forms that document the transport of these samples from the CPG field facility to ESI are provided in Appendix F.

2.2.3 Field deviations

The collection and handling of the 2012 freshwater sediment toxicity reference samples in the field was completed as described in the Benthic QAPP Addendum No. 5 (Windward 2012), with the following exception:

- During the reconnaissance survey, 12 SQT sampling locations were changed from the original target locations because the original locations were either inaccessible by boat, or the substrate was too coarse (e.g., rocky) to obtain acceptable sediment grab samples. PMF No. 1 to the Benthic QAPP Addendum No. 5 (Windward 2012) was prepared to provide the rationale for this USEPA-approved location change and the revised coordinates (Appendix G).

2.3 TOXICITY LABORATORY METHODS

This section provides a summary of the testing requirements and methods used to conduct toxicity testing for the 24 freshwater SQT reference samples collected in the LPR above Dundee Dam. Two toxicity tests were conducted: the 28-day *Hyalella azteca* survival and growth test, and the 10-day *Chironomus dilutus* survival and growth test.

Upon arrival at ESI, the samples were inspected, and the characteristics (e.g., coarseness, presence of indigenous organisms, debris) and condition of each sample were documented. Samples were given a unique tracking number and logged into the laboratory tracking system. Following protocols established for the USEPA-approved Benthic QAPP (Windward 2009), samples were not sieved prior to use. Samples were stored at 4 ± 1°C with nitrogen head space until use and when archived. The laboratory reports are provided in Appendix D.

2.3.1 *Hyalella azteca*

The 28-day *H. azteca* sediment toxicity test was conducted according to American Society for Testing and Materials (ASTM) Method E 1706-05 (ASTM 2010) and USEPA Method 100.4 (USEPA 2000).

H. azteca were exposed to test and negative control sediment for 28 days. The negative control sediment (used for quality control purposes) was a formulated sediment prepared according to USEPA (2000) methods. The organic material in the formulated sediment consisted of organic detritus from the ESI's chironomid culture combined with disintegrating unbleached paper pulp. The test was conducted with 8 replicates per treatment, each containing 100 mL of sediment and 225 mL of overlying water. The overlying water was natural, fresh surface water collected from the upper portion of the Taylor River watershed in Hampton Falls, New Hampshire, mixed with moderately hard reconstituted water (USEPA 2000) in a 50:50 ratio.

The test was initiated by adding 10 6-day-old amphipods to each replicate. The test was performed at 23 ± 1°C with a photoperiod of 16L:8D. A two-volume renewal of overlying water was conducted once each day, and 1.0 mL of a mixture of yeast, trout chow, and alfalfa suspension was added to each test chamber daily after water renewal. If the presence of residual, surplus food was observed, it was removed during daily water renewal.

Prior to renewing the overlying water each day, water quality parameters (i.e., DO, pH, specific conductance, and temperature) were measured in a surrogate chamber.[9] Additional parameters (i.e., alkalinity, ammonia, and hardness) were measured in the overlying water on Days 0 (i.e., test initiation), 7, 14, 22, and 28 (i.e., test termination). The total organic carbon of the overlying water and the ammonia of the pore water were measured on Days 0 and 28. The recorded readings are provided in the laboratory data report (Appendix D).

Aeration was initiated in all test chambers on Day 1 after DO in one of the surrogate chambers had been recorded at a level below acceptable levels (i.e., 2.5 mg/L). Aeration was maintained in all test chambers throughout the remainder of the test period. See Section 3.2.2 for additional details.

On day 28, the test was terminated, and the number of surviving amphipods in each replicate was counted and recorded. Notations were made if there was evidence of reproduction (e.g., presence of small amphipods). The surviving amphipods from each replicate were dried at 104°C to constant weight and weighed to the nearest 0.01 mg. The total weight of the dried amphipods from each replicate was divided by the number of surviving amphipods to obtain an average dry weight per replicate. The test was deemed acceptable if mean survival in the negative control was ≥ 80%, and there was measurable growth in the negative control organisms (compared with weight at test initiation) (USEPA 2000). The methods of the *H. azteca* toxicity test are summarized in Table 2-1.

[9] The surrogate chamber was treated exactly as a test chamber with the addition of organisms and food, but was not used to determine endpoint data.

Table 2-1. Summary of methods for the *Hyalella azteca* toxicity test

Parameter	Condition or Regimen
Test type	whole-sediment toxicity test with renewal of overlying water
Test duration	28 days
Endpoints measured	survival and growth
Test temperature	23°C (± 1°C)
Illuminance	cool white fluorescent bulbs
Photoperiod	16:8 hour light:dark
Test chamber	400-mL glass beakers
Test sediment volume	100 mL
Overlying water volume	225 mL
Overlying water	natural surface water collected from the upper portion of the Taylor River watershed in Hampton Falls, New Hampshire, mixed with moderately hard reconstituted water (50:50 ratio)
Renewal of overlying water	two-volume water change conducted once daily using water distribution system
Control sediment	formulated sediment prepared according to USEPA methods (2000); source of organic material was chironomid culture organic detritus and disintegrating unbleached paper pulp
Test organism	*Hyalella azteca*
Test organism source	cultured by Aquatic Research Organisms, Hampton, New Hampshire
Test organism age	7 to 8 days old (hatch date of12/1/2012)
Number of organisms/chamber	10
Number of replicate chambers/sample	8
Feeding	1.0 mL of yeast/trout chow/alfalfa suspension daily after water renewal
Aeration	none, unless DO in overlying water fell below 2.5 mg/L
Test protocol	USEPA 600/R-99/064, ASTM E1706-05
Test acceptability	mean control survival ≥ 80% and measurable growth of control test organisms
Reference toxicant	cadmium

ASTM – American Society for Testing and Materials
C – Celsius
DO – dissolved oxygen
ppth – parts per thousand
USEPA – US Environmental Protection Agency

2.3.2 *Chironomus dilutus*

The 10-day *C. dilutus* sediment toxicity test was conducted according to ASTM Method E 1706-05 (ASTM 2010) and USEPA Method 100.4 (USEPA 2000), as summarized in Table 2-2.

Table 2-2. Summary of methods for the *Chironomus dilutus* toxicity test

Parameter	Condition or Regimen
Test type	whole-sediment toxicity test with renewal of overlying water
Test duration	10 days
Endpoints measured	survival and growth (AFDW and ash free dry biomass)
Test temperature	23°C (± 1°C)
Illuminance	cool white fluorescent bulbs
Photoperiod	16:8 hour light:dark
Test chamber	400-mL glass beakers
Test sediment volume	100 mL
Overlying water volume	225 mL
Overlying water	natural surface water collected from the upper portion of the Taylor River watershed in Hampton Falls, New Hampshire
Renewal of overlying water	two-volume water change conducted once daily using water distribution system
Control sediment	formulated sediment prepared according to USEPA methods (2000); source of organic material was chironomid culture organic detritus and disintegrating unbleached paper pulp
Test organism	*Chironomus dilutus*
Test organism source	cultured by Aquatic BioSystems, Fort Collins, Colorado
Test organism age	8 to 10 days old; ≥ 50% at least third-instar larvae (second-instar larvae on 12/5/2012)
Number of organisms/chamber	10
Number of replicate chambers/sample	8
Feeding	1.5 mL of 6-mg/L TetraMin flake fish food suspension
Aeration	none, unless DO in overlying water fell below 2.5 mg/L
Test protocol	USEPA 600/R-99/064, ASTM E1706-05
Test acceptability	mean control survival ≥ 70% and mean weight/surviving organism of 0.48 mg AFDW
Reference toxicant	cadmium

AFDW – ash-free dry weight
ASTM – American Society for Testing and Materials
C – Celsius
DO – dissolved oxygen
ppth – parts per thousand
USEPA – US Environmental Protection Agency

C. dilutus were exposed to test and negative control sediment for 10 days. The negative control sediment (used for quality control purposes) was a formulated sediment prepared according to USEPA (2000) methods. The organic material in the formulated sediment consisted of organic detritus from the ESI's chironomid culture combined with disintegrating unbleached paper pulp. The overlying water was natural, fresh surface water collected from the upper portion of the Taylor River watershed in Hampton Falls, New Hampshire.

The test was conducted with 8 replicates per treatment, each containing 100 mL of sediment and 225 mL of overlying water. The test was initiated by adding 10 second- and third-instar larvae to each replicate. The test was performed at 23 ± 1°C with a photoperiod of 16L:8D. A two-volume renewal of overlying water was conducted once each day, and 1.0 mL of a 6-mg/L suspension of TetraMin flake fish food was added to each test chamber daily, after water renewal. If the presence of residual, surplus food was observed, it was removed during daily water renewal.

Prior to renewing the overlying water each day, water quality parameters (i.e., DO, pH, specific conductance, and temperature) were measured in a surrogate chamber. Additional parameters (i.e., alkalinity, ammonia, and hardness) were measured in the overlying water at test initiation and termination. The recorded readings are provided in the laboratory data report (Appendix D).

Aeration was initiated in all test chambers on Day 1 after DO in one of the surrogate chambers had been recorded at a level below acceptable levels (i.e., 2.5 mg/L). Aeration was maintained in all test chambers throughout the remainder of the test period. See Section 3.2.2 for additional details.

Test chambers were checked daily for pupation and emergence, and the number of emerged individuals was counted and recorded.

On day 10, the test was terminated, and the number of surviving organisms (i.e., larvae, pupae, and adult [emerged]) in each replicate was counted and recorded. The surviving larvae from each replicate (pupae and adult organisms were not included in the growth determination) were dried at 104 C to constant weight and weighed to the nearest 0.01 mg. The total weight of the dried larvae from each replicate was divided by the number of surviving larvae to obtain an average dry weight per replicate. The dried larvae were then ashed at 550°C for 2 hours. The ashed larvae were reweighed, and the tissue mass of the larvae was calculated as the difference between the weight of the dried larvae and the weight of the ashed larvae. Pupae and adult organisms were not included in the replicate to estimate ash-free dry weight (AFDW). The weight endpoint was based on the AFDW measurements. The test was deemed acceptable if mean survival in the negative control was ≥ 70%, and the mean weight of surviving negative control organisms was ≥ 48 mg AFDW (USEPA 2000).

2.3.3 QA/QC of toxicity tests

The sediment toxicity tests incorporated standard QA/QC procedures for evaluating the validity of the test results according to ASTM (ASTM 2010) and USEPA (2000) guidelines. Standard QA/QC procedures included the use of negative and positive controls and the periodic measurement of water quality during testing. The laboratory technicians performing the tests were responsible for ensuring that appropriate procedures were followed while conducting the tests. The laboratory performed the first data reduction by calculating average survival, dry weight (total weight divided by surviving number of organisms for each replicate), and dry biomass (total weight

divided by initial number of organisms for each replicate) for each test sediment sample and negative control sample. An internal review of the data was performed by the laboratory's QA / QC officer.

2.3.4 Validation methods

Paul Dinnel of Dinnel Marine Resources (DMR), an independent third party, conducted a validation of the toxicity data provided by ESI. By comparing the raw data with the electronic database and written test report, 100% of the data were validated. Any transcription errors, incorrect formulas, or other inconsistencies in the reports were corrected by ESI and verified by the independent reviewer before data were finalized. Further details on the data validation process are presented in Appendix E.

3 Results

This section presents the results of the toxicity tests conducted using the freshwater SQT reference samples collected above Dundee Dam. Section 3.1 provides results from the laboratory tests for *H. azteca* and *C. dilutus*, as well as the results of the negative control for both species. The results of the sediment toxicity test data validation are provided in Section 3.2. A summary of the data is provided in Appendix C. Laboratory reports are provided in Appendix D, and the validation report provided by DMR is presented in Appendix E.

3.1 SEDIMENT TOXICITY TEST RESULTS

Negative control performance was evaluated for both the *H. azteca* and *C. dilutus* tests, and was determined to be acceptable following USEPA and ASTM test acceptability criteria (ASTM 2010; USEPA 2000). The details for both *H. azteca* and *C. dilutus*, including the negative control results, are discussed in Sections 3.1.1 and 3.1.2, respectively.

3.1.1 *Hyalella azteca* toxicity test

The 28-day *H. azteca* sediment toxicity test using the 24 freshwater SQT reference samples was initiated on December 7, 2012, and included an evaluation of both survival and growth endpoints.

Mean negative control survival was 90%, which is acceptable based on the USEPA test acceptability criterion of mean control survival of at least 80% (USEPA 2000). The mean dry weight of 0.625 mg per surviving amphipod in the negative control is also considered acceptable based on the USEPA test acceptability criterion requiring measurable growth of control test organisms (USEPA 2000). The weight of a subset of organisms at test initiation was 0.015 mg / amphipod.

Results for the *H. azteca* test conducted using the 24 SQT reference sediment samples are presented in Table 3-1. Survival results are presented on Figure 3-1. Mean survival ranged from 0.0% at UPRT19J to 90.0% at UPRT18J. Mean weight ranged from 0.070 mg per surviving individual at UPRT20F to 0.531 mg per surviving individual at UPRT21G. Mean biomass, which was calculated by dividing total weight at the end of the test by the number of individuals at the start of the test, ranged from 0.0 mg at UPRT19J to 0.346 mg at UPRT19K.

Table 3-1. Summary of *Hyalella azteca* reference sediment toxicity test results

	Survival (%)		Weight[a] (mg)		Biomass[b] (mg)	
Location ID	Mean	St Dev	Mean	St Dev	Mean	St Dev
UPRT18H	85	13	0.311	0.073	0.264	0.0723
UPRT18I	73	26	0.253	0.0648	0.177	0.071

Table 3-1. Summary of *Hyalella azteca* reference sediment toxicity test results

Location ID	Survival (%)		Weight[a] (mg)		Biomass[b] (mg)	
	Mean	St Dev	Mean	St Dev	Mean	St Dev
UPRT18J	90	5.3	0.334	0.0624	0.302	0.0643
UPRT18K	75	15	0.376	0.0859	0.279	0.069
UPRT19J	0	0	na[c]	na[c]	0.0	0.0
UPRT19K	89	9.9	0.385	0.0616	0.346	0.0904
UPRT19L	65	21	0.341	0.134	0.232	0.131
UPRT19M	44	23	0.345	0.0315	0.148	0.0736
UPRT20A	60	30	0.252	0.0722	0.146	0.0809
UPRT20B	75	28	0.351	0.121	0.243	0.108
UPRT20C	76	21	0.394	0.115	0.306	0.135
UPRT20D	74	29	0.318	0.123	0.242	0.121
UPRT20E	66	14	0.337	0.114	0.226	0.0922
UPRT20F	1.3	3.5	0.070	na[d]	0.0009	0.0025
UPRT20G	68	15	0.291	0.127	0.199	0.105
UPRT21A	69	18	0.321	0.0882	0.218	0.0663
UPRT21B	19	19	0.201	0.124	0.0488	0.068
UPRT21C	78	7.1	0.353	0.0487	0.273	0.0335
UPRT21D	63	25	0.275	0.113	0.176	0.103
UPRT21E	58	22	0.269	0.101	0.159	0.0923
UPRT21F	73	17	0.343	0.0717	0.254	0.0909
UPRT21G	63	28	0.531	0.456	0.253	0.0969
UPRT22A	80	12	0.355	0.0732	0.29	0.0996
UPRT22B	59	20	0.458	0.128	0.265	0.101

[a] Weight is the total weight for each replicate divided by the number of survivors.

[b] Biomass is the total weight for each replicate divided by the initial number of organisms introduced into the test chamber.

[c] Weight data are not available for UPRT19J because there were no survivors.

[d] Standard deviation cannot be calculated for UPRT20F because only one replicate had survivors.

ID – identification

na – not applicable

St Dev – standard deviation

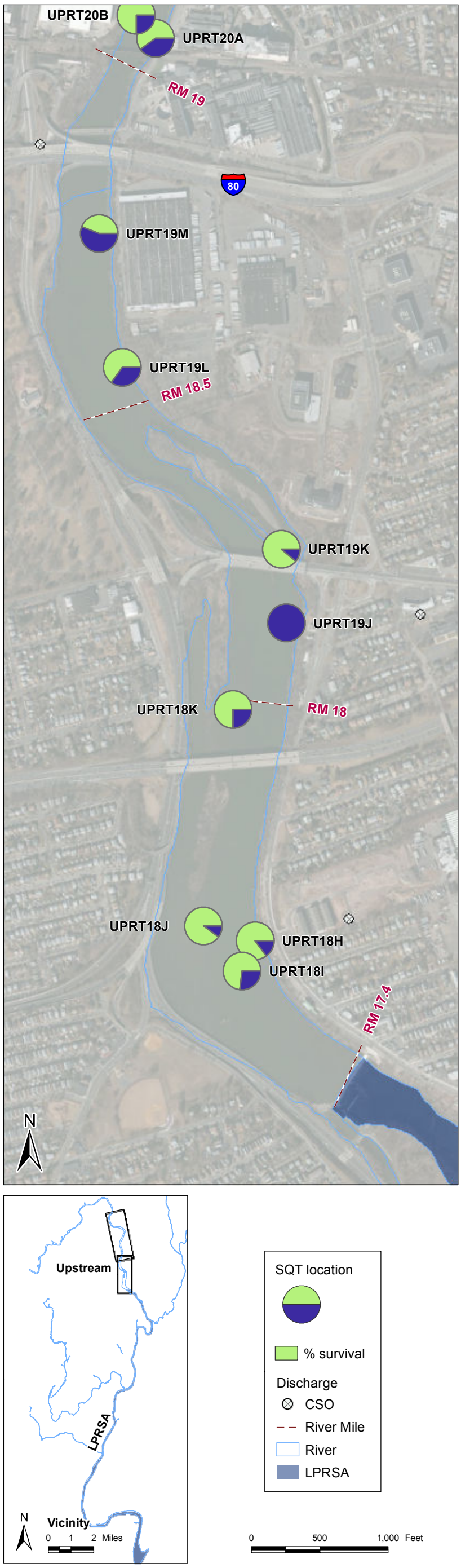


Figure 3-1. *Hyalella azteca* survival at SQT locations above Dundee Dam

Data sources: river miles were derived from USGS NHD Streams.
Imagery from ESRI; http://goto.arcgisonline.com/maps/World_Imagery

Prepared by LSM 9/26/2013; W:\Projects\06-58-01 Passaic RI\Data\GIS\Maps_and_Analysis\Upstream\5584_UPR Hyalella bioassay results_LSM_20130626.mxd

3.1.2 *Chironomus dilutus* toxicity test

The 10-day *C. dilutus* sediment toxicity test using the 24 freshwater SQT reference samples was initiated on December 7, 2012, and included an evaluation of both survival and growth endpoints.

Mean negative control survival was 98%, which is acceptable based on the USEPA test acceptability criterion of mean control survival of at least 70% (USEPA 2000). The mean AFDW of 1.88 mg per surviving larvae in the negative control is also considered acceptable based on the USEPA test acceptability criterion of 0.48 mg per surviving larvae (USEPA 2000).

Results for the *C. dilutus* test conducted using the 24 SQT reference sediment samples are presented in Table 3-2. Survival results are presented on Figure 3-2. Mean survival ranged from 3.8% at UPRT19J to 94% at UPRT18H. Mean weight (measured as AFDW) ranged from 1.21 mg per surviving larvae at UPRT20F to 2.23 mg per surviving larvae at UPRT21B and UPRT21F. Mean biomass (measured as AFDW and calculated by dividing the total AFDW by the number of individuals at the start of the test less the number of organisms that pupated or emerged during the testing period) ranged from 0.0 mg at UPRT19J to 1.87 mg at UPRT21F.

Table 3-2. Summary of *Chironomus dilutus* reference sediment toxicity test results

Location ID	Survival (%)[a]		Weight (mg)[b]		Biomass (mg)[c]	
	Mean	St Dev	Mean	St Dev	Mean	St Dev
UPRT18H	94	5.2	1.55	0.392	1.41	0.314
UPRT18I	71	24	1.71	0.329	1.17	0.366
UPRT18J	89	14	1.54	0.234	1.36	0.417
UPRT18K	86	7.4	1.47	0.0796	1.25	0.156
UPRT19J	3.8	11	na[d]	na[d]	0	0
UPRT19K	74	24	1.69	0.172	1.17	0.328
UPRT19L	78	13	1.64	0.26	1.26	0.284
UPRT19M	80	15	1.69	0.356	1.34	0.153
UPRT20A	79	20	1.91	0.486	1.38	0.318
UPRT20B	80	16	1.95	0.469	1.48	0.437
UPRT20C	90	5.3	1.77	0.286	1.55	0.316
UPRT20D	79	23	1.58	0.427	1.16	0.311
UPRT20E	85	16	1.64	0.226	1.36	0.328
UPRT20F	54	17	1.21	0.423	0.651	0.365
UPRT20G	85	12	1.76	0.357	1.49	0.419
UPRT21A	89	8.3	1.70	0.473	1.44	0.343
UPRT21B	79	9.9	2.23	0.576	1.66	0.446

Table 3-2. Summary of *Chironomus dilutus* reference sediment toxicity test results

Location ID	Survival (%)[a]		Weight (mg)[b]		Biomass (mg)[c]	
	Mean	St Dev	Mean	St Dev	Mean	St Dev
UPRT21C	73	8.9	1.96	0.387	1.24	0.335
UPRT21D	71	17	2.07	0.329	1.31	0.199
UPRT21E	84	14	1.64	0.164	1.30	0.424
UPRT21F	88	8.9	2.23	0.365	1.87	0.366
UPRT21G	83	23	1.71	0.404	1.29	0.383
UPRT22A	70	21	1.97	0.56	1.16	0.228
UPRT22B	81	11	1.76	0.237	1.36	0.210

[a] Percent survival is calculated using numbers of surviving larvae, pupae, and adults (emerged individuals).

[b] Weight is calculated as the total AFDW for each replicate divided by the number of surviving larvae.

[c] Biomass is calculated as the total AFDW for each replicate divided by the initial number of organisms introduced into the test chamber minus the number of organisms that either emerged or pupated during the test.

[d] No weight or biomass data are available for UPRT19J because only one replicate had any survivors, and the weigh pan for that replicate was dropped before it was weighed.

AFDW – ash-free dry weight
ID – identification
na – not applicable
St Dev – standard deviation

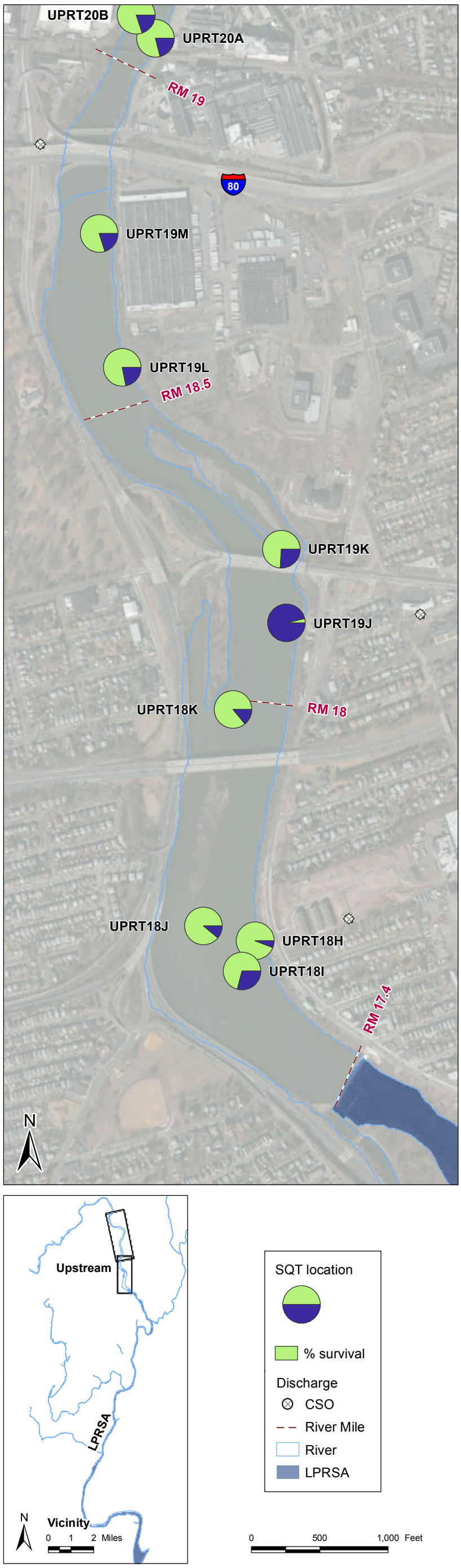


Figure 3-2. *Chironomus dilutus* survival at SQT locations above Dundee Dam

Data sources: river miles were derived from USGS NHD Streams.
Imagery from ESRI; http://goto.arcgisonline.com/maps/World_Imagery

Prepared by LSM 6/27/2013; W:\Projects\06-58-01 Passaic RI\Data\GIS\Maps_and_Analysis\Upstream\5583_UPR Chironomus bioassay results_LSM_20130626.mxd

3.2 QUALITY ASSURANCE/QUALITY CONTROL RESULTS

This section describes the results of the standard QA / QC procedures used to evaluate the quality of the sediment toxicity test data. Section 3.2.1 presents the QA / QC procedures conducted by the toxicity testing laboratory, and Section 3.2.2 presents a summary of the validation performed by DMR.

3.2.1 Laboratory QA/QC

The standard QA data provided by the laboratory included acceptable negative and positive control performance. As described in Section 3.1, the negative controls for both species met the test acceptability criteria (Tables 2-1 and 2-2) established for each test method (ASTM 2010; USEPA 2000).

The positive control consisted of a 96-hr reference toxicant test conducted with the same batch of test organisms as those used in the sediment toxicity tests, and using cadmium as the reference toxicant. Positive control results for both *H. azteca* and *C. dilutus* were acceptable. LC50 (concentration that is lethal to 50% of an exposed population) values for the positive controls conducted for both batches of test organisms fell within ± 2 standard deviations of the laboratory's historical mean value, indicating that the test organisms responded as anticipated to the known toxicant. The positive control results are provided in the laboratory reports in Appendix D.

The 28-day *H. azteca* test was initiated using 6-day-old amphipods rather than the recommended 7-to-14-day-old amphipods (USEPA 2000) (see Section 3.2.2 for a discussion of this performance criterion). Hardness, alkalinity, and ammonia in the overlying water did not vary by more than 50% during the test.

The 10-day *C. dilutus* test was initiated using second- and third-instar larvae as recommended by USEPA (2000). In addition, overlying water quality parameters for hardness, alkalinity, and ammonia did not vary by more than 50% during the test, with the exception of ammonia in one sample (UPRT19J) in which total ammonia increased from 0.21 to 0.58 mg / L. This concentration of total ammonia is low, and the increase is not expected to stress the test organisms.

As discussed in Section 3.2.2, on Day 0 in both the *H. azteca* and *C. dilutus* tests, the DO concentration in the overlying water was below the USEPA-recommended lower limit of 2.5 mg / L (USEPA 2000). This DO measurement was collected prior to the addition of organisms to the chambers. Aeration was started in all test chambers and was maintained above the 2.5 mg / L for the duration of the test periods.

3.2.2 Data validation

DMR performed validation of 100% the ESI toxicity test data. This validation included an initial evaluation of all data for completeness and accuracy, followed by a final evaluation of the overall quality and usability of the data. Validation was conducted by reviewing all raw data forms and electronic files, and noting any errors, omissions, or

discrepancies. Electronic files were checked to ensure that calculation formulas were correct. Any transcription errors, incorrect calculations, or other inconsistencies were corrected by ESI before the data were finalized.

A validation report summarizing the results of the QA review of the reference sediment test data generated by ESI is provided in Appendix E. This report includes a description of the laboratory facility based on an on-site audit conducted by DMR in March 2009.

The validation determined that most of the data generated from the toxicity testing of the reference sediment collected above Dundee Dam are of good quality and usable as toxicity data for the upstream reference area. The final QA evaluation noted the following:

- COC procedures were properly implemented, and no deviations were noted in sample transport or sample temperature.
- All tests were initiated within the eight-week sample hold time.
- Negative control test acceptability criteria were met for both tests.
- Positive control results were acceptable; LC50 values were within ± 2 standard deviations of the laboratory's control chart average LC50 values for each test.
- Data completeness was nearly 100% for the *H. azteca* test and 92.5% for the *C. dilutus* test.

The validation indicated a few protocol and water quality deviations from the laboratory SOPs attached to the Benthic QAPP (Windward 2012). The following is a list of the deviations:

- The *H. azteca* test was initiated with 6-day-old organisms based on the availability of test organisms the day the test was initiated. ESI's SOP states (on page 4) that tests will be initiated with 7- to 8-day-old organisms, following guidelines in USEPA (2000) protocol. The supplier did not have a sufficient quantity of organisms in the 7- to 8-day-old age range available during the week established for test initiation. Rather than initiate the tests on the weekend or use older organisms during the following week, the test was initiated on a Friday, when the organisms were 6 days old. The validator determined that the protocol deviation likely did not affect test results, because control performance met test acceptability criteria for both survival and growth endpoints. The data are, therefore, considered usable for the purposes of this study.
- An incorrect number of organisms were added to 4 test chambers during the *C. dilutus* test, based on the recovery of 11 organisms from each of those chambers at the end of the test period. The incorrect number of organisms was added to one replicate from three different samples (UPRT 20D, UPRT21F, and UPRT22A) and the control. However, because the statistical analyses conducted by the laboratory took into account the discrepancies in the initial counts when

they occurred, and because few replicates were affected, the validator has determined that interpretation of results should not be affected by the error. The data are, therefore, considered usable as toxicity data for the upstream reference area.

- During the *C. dilutus* test, 15 weigh pans were accidentally dropped before weight data could be obtained. The loss of the weight data was distributed across 13 samples; in 11 samples (UPRT18I, UPRT18K, UPRT19J, UPRT19L, UPRT19M, UPRT20F, UPRT20G, UPRT21B, UPRT21C, UPRT21d, and UPRT21G), 1 of the 8 replicates was lost, and in 2 samples (UPRT18H, UPRT20C), 2 of the 8 replicates were lost. The validator determined that the loss of 1 to 2 replicates for the 13 samples resulted in a minor reduction in statistical power for the analysis of weight and biomass for those samples. The data are, therefore, considered usable as toxicity data for the upstream reference area.
- Test chambers in the *C. dilutus* test were provided with 1.0 mL of 6 g/L Tetramin flake food daily, rather than 1.5 mL as specified in ESI's SOP. However, USEPA (2000) protocol recommends adding 1.5 mL of 4.0 g/L Tetramin daily to each test chamber, which is equal to 1.0 mL of 6 g/L Tetramin. Therefore, although the test procedure deviated from ESI's SOP, it did not deviate from USEPA protocol.
- Water quality deviations occurred during both the *H. azteca* and *C. dilutus* tests: the recorded temperature in the test chambers fell below the mean specified range of 23 ± 1°C, and exceeded the maximum fluctuation range of 23 ± 3°C at various times during the testing period, in particular during the first three days of testing. The validator determined that the low temperatures during the early part of the tests may have slightly reduced amphipod and chironomid larval growth, but that temperatures were within tolerance ranges (0 to 33°C for *H. azteca* and 0 to 35°C for *C. dilutus*) and control growth was acceptable in both tests. Final results were not likely compromised by the low temperatures. The data are, therefore, considered usable as toxicity data for the upstream reference area.
- On Day 0, prior to introduction of organisms in both the *H. azteca* and *C. dilutus* tests, the DO concentration for one sample (UPRT20B) was below the lower limit of 2.5 mg/L specified in the protocol (USEPA 2000). Aeration was immediately initiated in all test chambers, and was maintained throughout the remainder of the test period.
- On Day 0, ammonia concentrations in sediment porewater and overlying water for sample UPRT21B were elevated compared to concentrations in the rest of the samples. The validator noted that the ammonia concentrations in UPRT21B were below the four-day LC50 concentration for both the *H. azteca* and *C. dilutus* tests, but also noted the possibility that organisms exposed to this sample may have been stressed by ammonia. However, unionized ammonia levels were below the

0.4-mg/L threshold that would trigger purging ammonia prior to the introduction of organisms.

4 Summary

The objectives of the 2012 reference sediment collection program were met with regard to the collection of SQT samples and the toxicity testing of sediments collected above Dundee Dam. These data will be used to establish a reference envelope to assist in understanding LPRSA sediment toxicity test results that will be presented in the BERA.

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