Monitoring the Tidal Delaware River for Ambient Toxicity
2012 Narrative Report

Submitted to U.S. Environmental Protection Agency—Region III

Delaware River Basin Commission

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1.0 SUMMARY

This report is an update on studies to assess ambient waters in an urbanized area of the tidal Delaware River. The objective of the 2012 survey was to determine whether chronic lethal or sublethal toxicity, as measured in laboratory experiments, was present in river water samples. Toxicity at sixteen fixed stations in the main stem of the tidal Delaware River with salinities from 0 to 15 parts per thousand (ppt) was assessed using six species: *Pimephales promelas*, *Americamysis bahia, Menidia beryllina, and Ceriodaphnia dubia* in 7-day tests*;* *Pseudokirchneriella subcapitata* in a 96-hour test*;* and *Hyalella azteca* in a 10-day water-only test. Endpoints appropriate for each test method including survival, growth, and reproduction were measured in the toxicity tests. Based on the measured endpoints, eleven sites in the main stem of the Delaware River clearly did not indicate chronic toxicity to the tested species. Results for ambient samples from three sites, between River Mile 63 and 70, indicated effects on growth of one species, the mysid *A. bahia*, while results for another site, at River Mile 55, indicated effects on both survival and growth of *A. bahia*. It should be noted, however, that toxicity tests with *A. bahia* involved the use of salinity adjusted test samples and acclimated test organisms. Therefore, results from bioassays with *A. bahia* should be interpreted in the context of results from assays with other test species use in the study. One freshwater site T6 at River Mile 75.1 indicated effects on growth on the alga *P. subcapitata*. However, results at site T6 may not indicate significant adverse effects because algal growth exceeded acceptable test criteria. The area of the river in DRBC Water Quality Zone 5 near sites T2, T3, T4, T5 and T6 warrants further assessment to confirm the existence and persistence of toxicity and to evaluate potential sources (chemical causes) of observed toxicity.

2.0 INTRODUCTION

The Delaware River Port Complex (including docking facilities in Pennsylvania, New Jersey, and Delaware) is the largest freshwater port in the world. The tidal portion of the Delaware River, where most of this commerce occurs and where approximately six million people live, was the study area for an investigation of ambient toxicity. This area is designated at Water Quality Management Zones 2 through 5 by the Delaware River Basin Commission (DRBC) (Figure 1).

The Delaware River and Bay is a vital ecosystem with habitat for numerous species of finfish, as well as clams, oysters, and crabs. It supports the second largest concentration of migrating shorebirds in the Western Hemisphere, contains habitat for many different species of waterfowl, and is the principal breeding grounds for the American horseshoe crab (Delaware Estuary Program, 1995). Potential sources of toxicity and water quality impairment in the Delaware Estuary include point and non-point sources, contaminated sites, tributaries, the Chesapeake and Delaware Canal, atmospheric deposition and contaminated sediment (Delaware Estuary Program, 1996). Fish consumption advisories are in place for segments of the study area due to existing concentrations of polychlorinated biphenyl (PCB), dioxins, furans, mercury and chlorinated pesticides (DRBC, 2010). In addition, sediment toxicity, elevated contaminant levels in sediment, and degraded benthic communities have been observed within the study area (Costa and Sauer, 1994; Hartwell and Claflin, 2005; Hall *et al*., 2005; McCoy *et al*., 2002; USACE, 2012; and USEPA, 2004). Based on existing water quality regulations for the estuary, no adverse effects should be observed in toxicity tests with undiluted ambient water (DRBC, 2012; USEPA, 1991). In 2000, the DRBC determined that the assimilative capacity of Zones 2 - 5 was exceeded for chronic toxicity and recommended continued monitoring to assess the cumulative

effect of toxicity sources. Monitoring toxicity is therefore an essential component of programs designed to protect this valued resource. The objective of this study was to assess the potential for chronic lethal or sublethal toxicity in water samples collected from sampling stations in the tidal Delaware River.

A number of programs monitor chemical contaminants and toxicity in permitted wastewater discharges, water, sediment and benthic organisms in the Delaware Estuary (http://www.delawareestuary.org/science_projects_baybottom.asp, USEPA, 2004). Since the DRBC monitoring program is the only on-going program to test for water column toxicity in the estuary, a cooperative effort was initiated by the DRBC through the formation of an Ambient Toxicity Workgroup to develop a scientifically sound sampling and analysis plan, with a holistic, broad, long-term view, to determine whether ambient toxicity occurs in the waters of the estuary. The Ambient Toxicity Workgroup includes personnel from the DRBC, U.S. Environmental Protection Agency (USEPA), basin states, municipal agencies, industry, and other interested parties. The Workgroup reviews and provides input on project plans for ambient toxicity monitoring as well as reviewing and commenting on the results from the toxicity testing.

3.0 MATERIALS AND METHODS

3.1 Selection of Test Species

Toxicity in Delaware Estuary waters is assessed with standard test species used for testing effluents under the USEPA NPDES program; the same species have frequently been used to monitor receiving water toxicity (USEPA, 2002a and USEPA, 2002b). Three freshwater species

were selected, for waters with conductivity ≤ 1750 µmhos/cm or ≤ 1 ppt salinity at 25 °C, a fish, *Pimephales promelas* (fathead minnow); an invertebrate, *Ceriodaphnia dubia* (water flea); and a green alga, *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*).

Several of the sampling sites selected experienced changes in salinity due to river flow and tidal conditions. The selection of test species and appropriate controls was complicated by this changing salinity gradient. For water samples with salinity >1 ppt, additional test species were selected that were tolerant of salinity (1 to 15 ppt) and met the prescribed test acceptability requirements at ambient salinities. The species also had to be a standard toxicity test species and commercially available. The three salinity tolerant species selected were a mysid, *Americamysis bahia* (formerly *Mysidopsis bahia*); a fish, *Menidia beryllina* (inland silverside); and an amphipod, *Hyalella azteca*. Acclimation of *A. bahia* to lower salinities during culturing prior to testing was needed to obviate or limit the need for major salinity adjustment of river water samples from as low as 1 ppt to the standard test conditions at 20 ppt. *A. bahia* have been reported to meet test acceptance criteria in 7- and 28-d toxicity tests when tests were conducted with salinity as low as 10 ppt (Ward et al., 2006). MacGillivray *et al*. (2011) provide additional information on species selection and acclimation of mysids to lower salinity as well as results from DRBC monitoring in the Delaware Estuary from 2005 through 2009.

3.2 Study Design

Evaluations of all sampling sites were made with tests using 100% ambient water. Results from these tests were compared to controls of reconstituted laboratory water formulated to mimic freshwater (salinity < 1 ppt) and brackish water (salinities of 5, 10, 15 or 25 ppt). Water samples

were taken from sixteen sites in the mainstem of the tidal Delaware River. Figure 1 is a map of the tidal Delaware River indicating the sampling sites. Sites were at or near locations routinely sampled for water quality, thus a long-term monitoring database for physical-chemical parameters is associated with sites sampled for toxicity (DRBC, 2004). The sampling was not designed to characterize any potential near-field toxicity issues immediately surrounding point source discharges or other contaminant sources. USEPA short-term chronic toxicity methods were used to evaluate toxicity and sublethal effects in ambient samples with *Pimephales promelas*, *Americamysis bahia, Ceriodaphnia dubia,* and *Menidia beryllina* in 7-day tests*; Pseudokirchneriella subcapitata* in a 96-hour test*;* and *Hyalella azteca* in a 10-day water-only test. Endpoints evaluated by these methods included survival, growth and reproduction (USEPA 2002a and USEPA 2002b). In the *H. azteca* tests an artificial substrate (Nylon coiled-web material) was used as a substrate and water was renewed daily (USEPA, 2000). Additional modifications to the toxicity test methods are described in the salinity adjustment and control section below.

At each main stem sampling site, a single grab sample was collected in the navigation channel for each location. All samples were collected at a depth of 0.6 of the water column using a 10 liter Niskin sampling bottle (Model 1010-1.2, General Oceanics, Miami, FL) configured to collect a vertical sample. Water was collected on three sampling days in 2012 (August 20, 22 and 24) corresponding to use of these samples on test days 1, 3, and 5, respectively. On each day of sampling, in-field measurements were made for specific conductivity, salinity, water temperature, dissolved oxygen and pH using a Hydrolab or other appropriate meters (Table 1). Water samples for toxicity testing were transported to the laboratory in LDPE plastic cubitainers

(VWR Int., Brisbane, CA) on ice in coolers to maintain the temperature at 4 ^{o}C ± 2 ^{o}C. Temperature inside the cooler was tracked during transport with a temperature logger.

3.3 Salinity Adjustments and Controls

In toxicity tests with salinity tolerant species *A. bahia*, *M. beryllina*, and *H. azteca*, the test salinity adjustment was based on the ambient salinity of the first sample collected at each site. If the ambient water salinity was lower on subsequent sampling days by greater than 2 ppt from the initial sample, the salinity was adjusted to the initial sample day conditions. No salinity adjustment was performed if salinity increased between sampling days. *A. bahia* was tested at ambient salinities when salinity was ≥ 10 ppt. If <10 ppt, the sample was adjusted to 10 ppt. The *A. bahia* tests included controls at salinities of 10 ppt, 15 ppt and 25 ppt. *Menidia beryllina* were tested at ambient salinities if the salinity was ≥ 5 ppt. If the ambient salinity was <5 ppt, the sample was adjusted to 5 ppt. The *M. beryllina* tests included controls at salinities of 5 ppt and 10 ppt. *Hyalella azteca* was tested at the ambient salinity up to 10 ppt. Ambient water for the *H. azteca* tests did not need salinity adjustment. *H. azteca* tests were conducted with three controls at salinities of 1 ppt, 5 ppt, 10 ppt and 15 ppt.

3.4 Hydrology and Tides

Low flow conditions were targeted for sampling to assess the effects of wastewater effluents on the tidal river and to be within the range of flows used to regulate contaminants in surface waters. The mean daily average flows for the Delaware River at Trenton, NJ were between 3,200 to 4,500 cfs on sampling days (August 20, 22 and 24, 2012) with the highest flow on the first day

of sampling. Flows in the Delaware River have been lower than 3,200 cfs less than 10% of the time and lower than 4,500 cfs less than 20% of the time in the period of record between 1980 and 2011. No significant precipitation was recorded during the sampling period of August 20 to 24, 2012. On August 18, 2012, two days prior to the sample event, 0.5 inches of rainfall was recorded in most areas draining to the sampling locations (http://water.weather.gov/precip).

Slack tide was targeted to facilitate sampling while tidal velocities are smaller. However, sampling occurred at different points in the tidal cycle at various sites in the sampling area. See sampling locations, dates, and times aligned with NOAA predicted tides and currents [http://tidesandcurrents.noaa.gov/ofs/dbofs/dbofs.html] (Figures 2, 3 and 4). Samples were generally collected closer to slack tide in the down river on all three days. For example, on August 22, 2012, sampling at sites T1, T2, T3, T5 and T6 occurred during an ebbing tide. Therefore, tidal flow was outgoing while sampling at those locations. Also on August 22, 2012, sampling at sites T11, T12, T15 and T16 occurred during a rising tide. Therefore, tidal flow was incoming while sampling at those locations. Other sites on August 22, 2012 were sampled during low slack tide when the tidal flow was neither incoming nor outgoing.

3.5 Statistical Analysis

Statistical comparisons were made between the salinity control closest to the ambient sample salinity at each test site. All statistical analysis followed USEPA guidance for each test method (USEPA 2002a and USEPA 2002b) using ToxCalc v5.0 software (Tidepool Scientific Software, McKinnleyville, CA USA). Values for the no-observed-effect concentration (NOEC) were calculated using, analysis of variance with Dunnett's test or the Bonferroni t test. An alpha level

of 0.05 was used for the hypothesis testing. Assumptions of normality were tested by Shapiro-Wilk's Test or Kolmogorov D Test with an alpha of 0.01. Homogeneity was tested by Bartlett's Test with an alpha of 0.01.

To assure that differences between controls and treatment were biologically significant as well as a statistically significant difference, a test was not considered positive for toxicity unless there was > 20 % difference observed between control and ambient water in the tests. In addition, a test for significant toxicity (TST) was conducted using results for 100% ambient water from sample sites compared to a control using the Welch's t test at recommended b value for chronic tests of 0.75 and alpha levels for *C. dubia* at $\alpha = 0.20$, *P. promelas*, *M. beryllina* and *P. subcapitata* at $\alpha = 0.25$ and *A. bahia* at $\alpha = 0.15$ (Shukla et al., 2000; USEPA, 2010). In the absence of recommended alpha values for *H. azteca*, the Welch's t test was not used with data from this species.

4.0 RESULTS AND DISCUSSION

Evaluation of the No Observed Effect Concentration (NOEC) for survival, growth and reproduction indicated the lack of significant chronic lethal or sublethal effects (NOECs = 100%) at 11 sites (T1, T7, T8, T9, T10, T11, T12, T13, T14, T15 and T16) while four sites (T2, T3, T4, T5 and T6) had significant chronic lethal or sublethal effects (NOEC < 100% for survival and/or growth in the mysid, *A. bahia*) based on a statistically significant difference from the control. No effects were observed when *P. promelas* or *C. dubia* were exposed to 100% surface water from any of the freshwater sites (T6 through T16) (Table 2). No effects were observed when *P. subcapitata* was exposed to 100% surface water from the freshwater sites T7 through T16 (Table

2). No effects were observed when *M. beryllina* or *H. azteca* were exposed to 100% surface water from any of the brackish sites (T1 through T5) (Table 3).

Although the freshwater site T6 had both a NOEC < 100%, with a reduction of 43.9%, and failed the TST for *P. subcapitata*, results at site T6 may not indicate significant adverse effects because the mean cells/ml of the green algal, *P. subcapitata* in the 100% surface water at $2.52x10^6$ cells/ml exceeded acceptable test criteria of 1 x 106 cells/ml (Table 2). Although sites T9 and T12 had NOEC = 100% for *P. subcapitata* growth when calculated using analysis of variance with the Bonferroni t test, both T9 and T12 failed the TST evaluation for *P. subcapitata* growth probably due to high within-test variability (Denton *et al,* 2011). In addition, the observed differences from the control at 18.55% for T9 and 19.77% for T12 did not meet the predetermined criteria of >20% difference from the controls for significant biological effect.

The ambient water with the greatest observed effect was sampled at site T2 (Reedy Island). Surface water exposed to *A. bahia* (salinity adjusted to 10 ppt) had a NOEC < 100% for both survival and growth based on a statistically significant difference from the 10 ppt control. Mean percent survival in site T2 water was 70% compared to survival in the 10 ppt control at 97.5%. Survival was 100% in both the 15 ppt and 25 ppt *A. bahia* controls. Measured *A. bahia* growth in site T2 water was 0.1640 mg mean dry weight compared to a mean dry weight in the 10 ppt control of 0.2608 mg which is a 37% effect. The mean dry weight of the 15 ppt and 25 ppt *A. bahia* controls were 0.2870 and 0.3403 mg, respectively. All controls met test acceptability criteria for the *A. bahia* test at 80% survival and ≥ 0.2 mg mean dry weight (U.S. Environmental Protection Agency, October 2002b) (Table 3).

Americamysis bahia tests (salinity adjusted to 10 ppt) with water from sites T3 (North of Pea Patch Island), T4 (South of Delaware Memorial Bridge) and T5 (North of Delaware Memorial Bridge) had NOECs = 100% for the survival endpoint but had NOECs < 100% for the growth endpoint based on a statistically significant difference from the 10 ppt control. The *A. bahia* growth measured as mean dry weight in the site waters fromT3 was 0.1818 mg (30% effect), from T4 was 0.1803 mg (31% effect), and from T5 was 0.1745 mg (33% effect) compared to a mean dry weight in the 10 ppt control of 0.2608 mg (Table 3).

An additional test for significant toxicity confirmed the NOEC results for tests with *A. bahia.* When data from *A. bahia* exposed to 100% ambient water versus a control at 10 ppt salinity were compared using the Welch's t test at a recommended b value for chronic tests of 0.75 and an alpha level for *A. bahia* of $\alpha = 0.15$, sites T2, T3, T4 and T5 indicated significant toxicity while site T1 did not indicate toxicity (Table 3).

5.0 CONCLUSIONS

The objective of this study was to determine the potential for chronic lethal or sublethal toxicity in ambient water samples collected from sampling stations in the tidal Delaware River. This survey consisted of water column toxicity tests on samples collected during a period of low river flow in August 2012. Six species were used in the study including *Pimephales promelas*, *Americamysis bahia*, *Menidia beryllina*, and *Ceriodaphnia dubia* in 7-day tests; Pseudokirchneriella subcapitata in a 96-hour test; and Hyalella azteca in a 10-day water-only test. Based on the measured endpoints appropriate for each test method including survival,

growth, and reproduction, testing of samples from eleven of sixteen sites in the main stem of the Delaware River did not indicate chronic toxicity to the tested species. Toxicity was observed in samples from four sites between River Mile 55 and 70. Effects in the growth endpoint of one species, *A. bahia*, was observed at three sites between River Miles 63 and 70. Effects on both survival and growth endpoints of *A. bahia* were observed in one site at River Mile 55. It should be noted, however, that toxicity tests with *A. bahia* involved the use of salinity adjusted test samples and acclimated test organisms. Therefore, results from bioassays with *A. bahia* should be considered with some scrutiny, and should be interpreted in the context of results from assays with other test species which indicated the lack of adverse effects. One freshwater site T6 at River Mile 75.1 had both a NOEC < 100%, with a reduction in algal growth of 43.9%, and failed the TST for *P. subcapitata*. However, results at site T6 may not indicate significant adverse effects because algal growth exceeded acceptable test criteria. The area of the river near sites T2, T3, T4, T5 and T6 warrants further assessment to confirm the magnitude and persistence of toxicity. Should such follow-up studies indicate toxicity, samples may be used in a toxicity identification evaluation procedure to evaluate potential sources (chemical causes) of toxicity.

6.0 ACKNOWLEGEMENTS

Results from this study were presented to and discussed by the DRBC Chronic Toxicity Workgroup members on January 16, 2013. This report was reviewed by and comments received from Dr. Thomas J. Fikslin of the DRBC, Dr. Steven S. Brown of Dow Chemical Company, Dr. Richard Greene of Delaware Dept. of Natural Resources, and Dr. Robert A. Hoke of E.I. duPont de Nemours. Sample site map (Figure 1) is courtesy of Karen Reavy (DRBC). Figures 2 to 4 are

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8.0 FIGURES AND TABLES

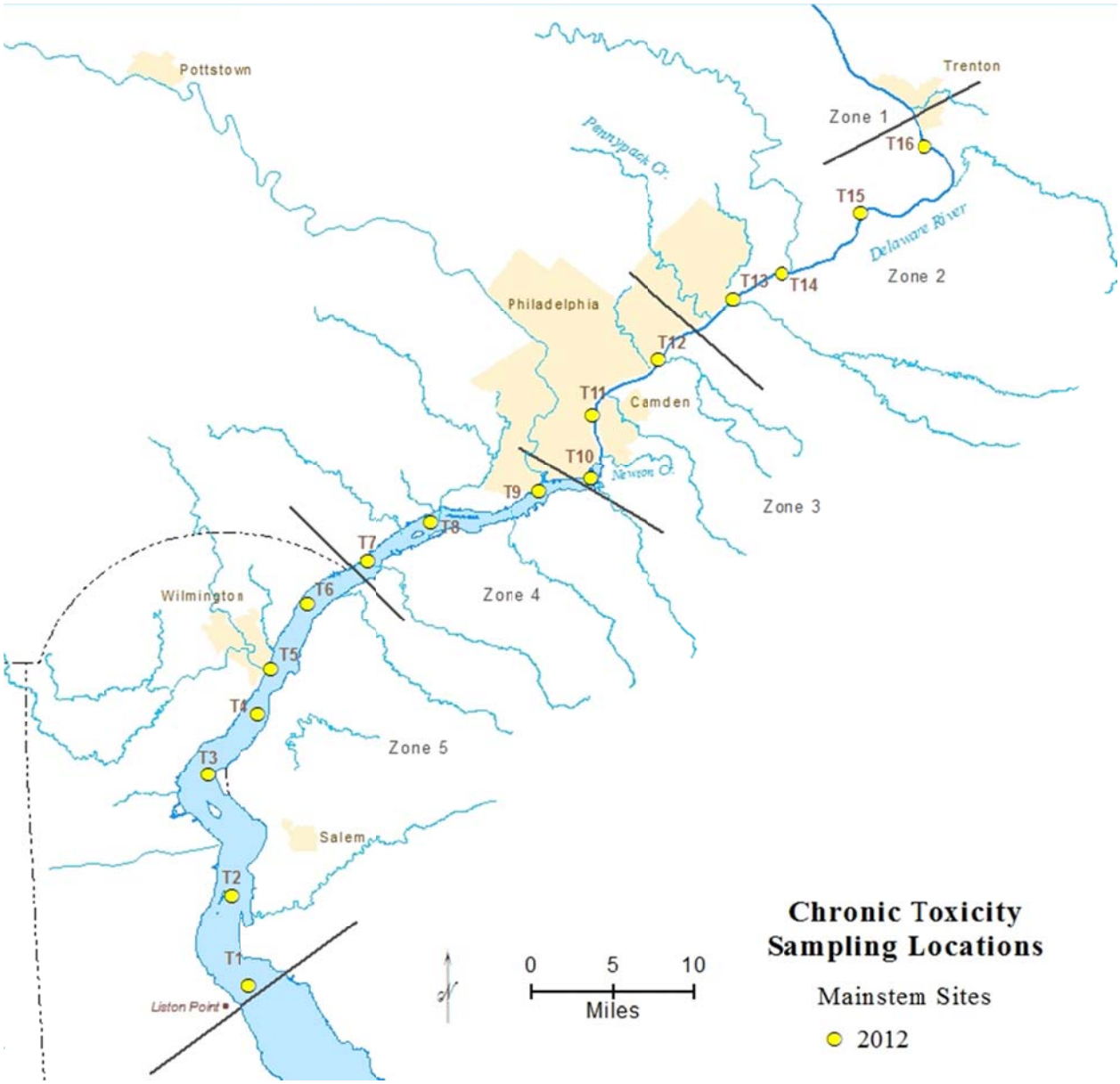

Figure 1. Sample sites in the tidal Delaware River

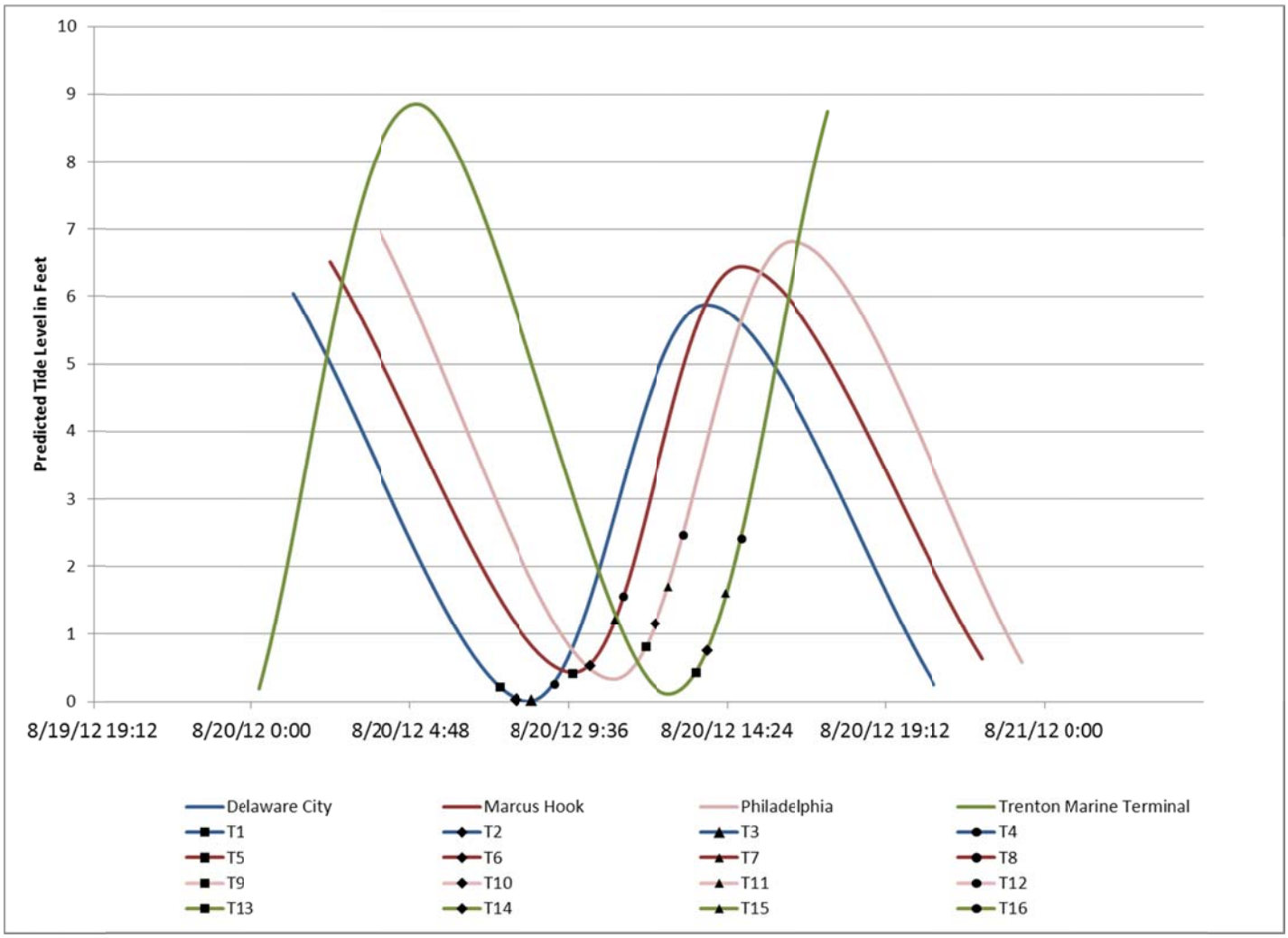

Figure 2. Tidal conditions at sampling sites on August 20, 2012

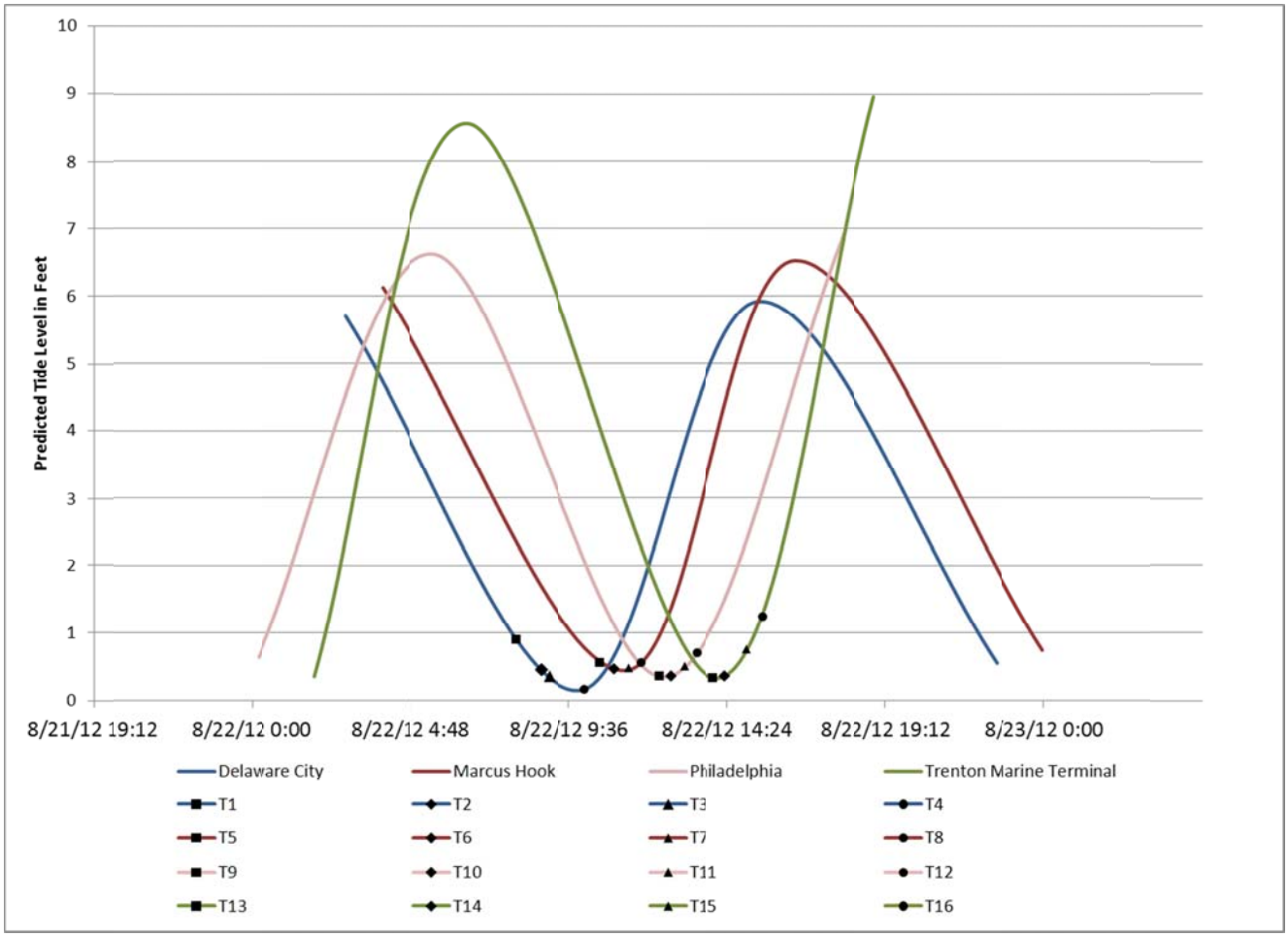

Figure 3. Tidal conditions at sampling sites on August 22, 2012

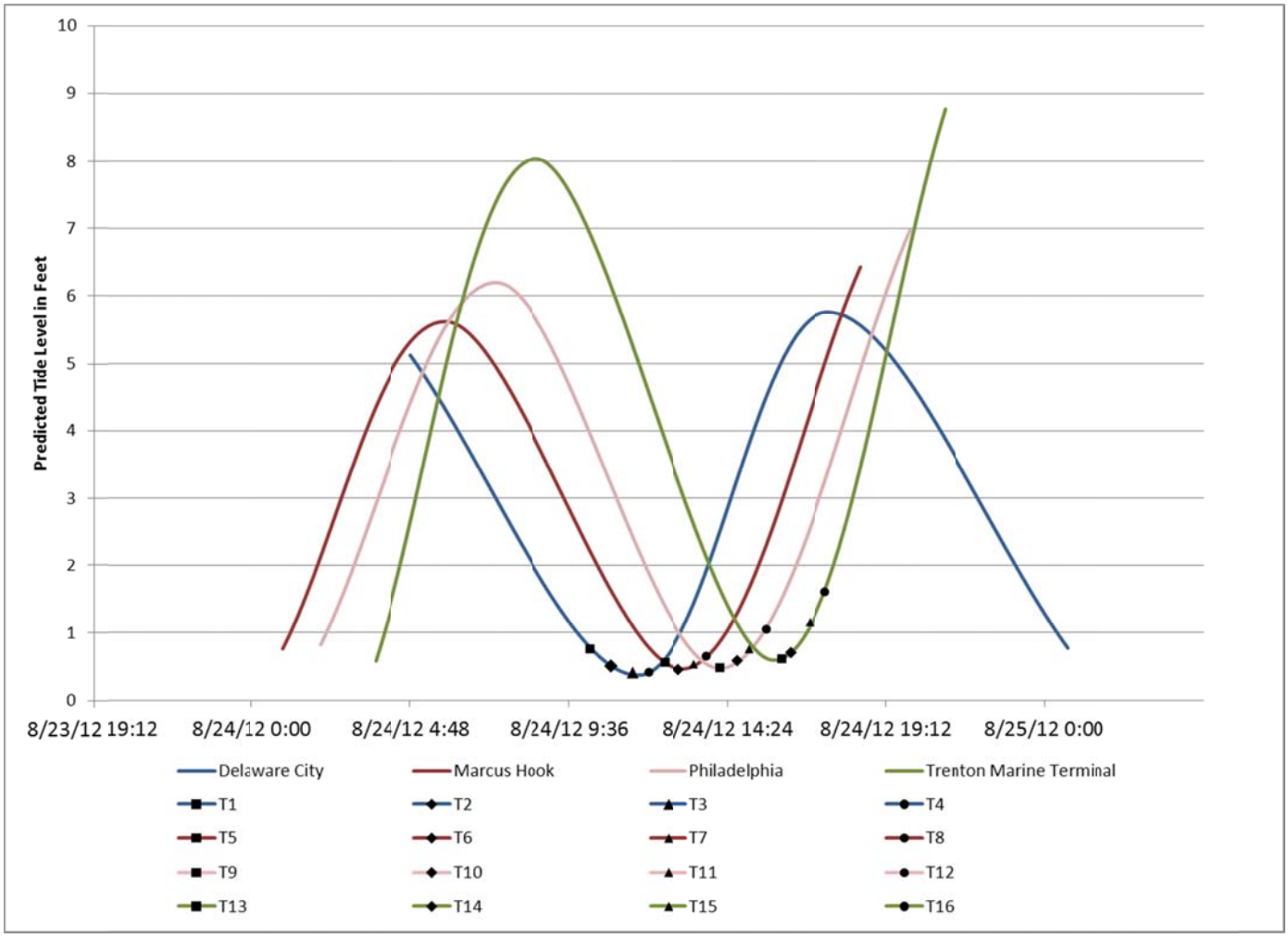

Figure 4. Tidal conditions at sampling sites on August 24, 2012

Table 1. Physical-chemical data in study area

Parameter	Maximum	Minimum	Median	Detection Limit
dissolved oxygen (mg/L)	11.2	3.8	5.8	0.1
pH	7.8	6.8	7.31	0.1
temperature (^{o}C)	27.7	23.4	26.8	NA
specific conductivity (µS/cm)	17,500	239	-	2
salinity (ppt)	9.5	0.8	-	0.1

Table 2. Short-term chronic toxicity tests with freshwater species

Zone	Site	River Mile/ Latitude Longitude	*P. promelas* **fish** survival and growth	*C. dubia* **invertebrate** survival and reproduction	*P. subcapitata* **algae** growth
5			**NOEC/TST**	**NOEC/TST**	**NOEC/TST**
	T6 Oldman's Pt	75.1 39°46.08′ 75°28.44′	100% Pass	100% Pass	<100%[1] Fail
4	T7 Marcus Hook Creek	80 39°48.94′ 75°23.62′	100% Pass	100% Pass	100% Pass
	T8 Eddystone	85 39°50.75′ 75°20.32′	100% Pass	100% Pass	100% Pass
	T9 South of Schuylkill River	90 39°52.56′ 75°12.16′	100% Pass	100% Pass	100% Fail[2]
3	T10 Big Timber Creek	95.5 39°53.20′ 75°08.39′	100% Pass	100% Pass	100% Pass
	T11 Penn's Landing	99.4 39°56.44′ 75°08.15′	100% Pass	100% Pass	100% Pass
	T12 Pennsauken Creek	105.4 39°59.58′ 75°03.66′	100% Pass	100% Pass	100% Fail[2]
2	T13 Rancocas Creek	111.5 40°02.88′ 74°58.55′	100% Pass	100% Pass	100% Pass
	T14 Beverly	115 40°04.23′ 74°55.55′	100% Pass	100% Pass	100% Pass
	T15 Florence	122 40°07.44′ 74°48.24′	100% Pass	100% Pass	100% Pass
	T16 Biles Channel	131.1 40°10.87′ 74°44.68′	100% Pass	100% Pass	100% Pass

[1] Means cells/ml - control $4.49 \times 10^{6,}$ sample 2.52×10^{6} exceeds test acceptability criteria mean of 1×10^{6} cells/ml

[2] Did not meeting predetermined criteria for significant biological effect. Single concentration short-term chronic tests at 100%aAmbient water.

Table 3. Short-term chronic toxicity test with salinity tolerant species

Zone	Site/ Initial Salinity	River Mile Latitude Longitude	*A. bahia* shrimp Survival and growth[1]	*M. beryllina* fish Survival and growth	*H. azteca* amphipod Survival and growth
			NOEC/TST	**NOEC/TST**	**NOEC[2]**
5	T1 Liston Pt. (9.5 ppt)	50 39°26.12′ 75°31.46′	100% Pass	100% Pass	100%
	T2 Reedy Island (6.7 ppt)	55 39°30.43′ 75°33.25′	<100% survival and growth 70% and 0.1640 mg Fail	100% Pass	100%
	T3 North of Pea Patch Island (4.2 ppt)	63 39°36.39′ 75°34.36′	<100% growth 0.1818 mg Fail	100% Pass	100%
	T4 South of Del Mem. Bridge (2.3 ppt)	68 39°40.28′ 75°31.64′	<100% growth 0.1803 mg Fail	100% Pass	100%
	T5 North of Del. Mem. Bridge (1.2 ppt)	70.8 39°43.06′ 75°30.42′	<100% Growth 0.1745 mg Fail	100% Pass	100%

[1] *A. bahia* were acclimated from salinity at 25 ppt to 10 ppt. Salinity of ambient water was adjusted upward to 10 ppt for testing. All controls met acceptable test criteria of 80% survival and ≥ 0.20 mg mean dry weight.

[2] TST was not calculated with data for *H. azteca*. Single concentration short-term chronic tests at 100% ambient water. NOEC = No Observed Effect Concentration; TST = Tests of Significant Toxicity