NBP-91-74

Report on Providence River Toxicity Testing:

Wet/Dry Study 67 pp

Morrison, G. (EPA-ERLN)

Narragansett Bay Estuary Program

PROVIDENCE RIVER TOXICITY TESTING
WET/DRY STUDY

Compiled by:

George Morrison

U.S. Environmental Protection Agency
Environmental Research Laboratory
Narragansett, Rhode Island

#NBP-91-74

FOREWORD

The United States Congress created the National Estuary Program in 1984, citing its concern for the "health and ecological integrity" of the nation's estuaries and estuarine resources. Narragansett Bay was selected for inclusion in the National Estuary Program in 1984 , and the Narragansett Bay Project (NBP) was established in 1985. Narragansett Bay was designated an "estuary of national significance" in 1988. Under the joint sponsorship of the U.S. Environmental Protection Agency and the Rhode Island Department of Environmental Management, the NBP's mandate is to direct a program of research and planning focussed on managing Narragansett Bay and its resources for future generations.

The NBP will develop a draft Comprehensive Conservation and Management Plan (CCMP) by December, 1991, which will recommend actions to improve and protect the Bay and its natural resources.

The NBP has established the following seven priority issues for Narragansett Bay:

- management of fisheries
- nutrients and potential for eutrophication
- impacts of toxic contaminants
- health and abundance of living resources
- health risk to consumers of contaminated seafood
- land-based impacts on water quality
- recreational uses

The NBP is taking an ecosystem/watershed approach to address these problems and has funded research that will help to improve our understanding of various aspects of these priority problems. The Project is also working to expand and coordinate existing programs among federal, state and local agencies, as well as with academic researchers, in order to apply research findings to the practical needs of managing the Bay and improving the environmental quality of its watershed.

This report represents the technical results of an investigation performed for the Narragansett Bay Project. The information in this document has been funded wholly or in part by the United States Environmental Protection Agency through Cooperative Agreement #CX812768 to the Rhode Island Department of Environmental Management. It has been subject to the Agency's and the Narragansett Bay Project's peer and administrative review and has been accepted for publication as a technical report by the Management Committee of the Narragansett Bay Project. The results and conclusions contained herein are those of the author(s), and do not necessarily represent the views or recommendations of the NBP.

This report is one component of a study coordinated by Dr. Raymond M. Wright: "Problem Assessment and Source Identification and Ranking of Wet Weather Discharges Entering the Providence and Seekonk Rivers". The interested reader is encouraged to investigate this reference for a comprehensive analysis of wet weather discharges from point and nonpoint sources of pollution in the Providence River drainage system.

NARRAGANSETT BAY PROJECT

REPORT ON

PROVIDENCE RIVER TOXICITY TESTING

WET/DRY STUDY

EXECUTIVE SUMMARY

A study was undertaken during the summer of 1989 to evaluate the toxicity of various point-source discharges into the Providence River and associated ambient waters. This study was a cooperative venture involving the Narragansett Bay Project, the Narragansett Bay Commission, the University of Rhode Island Department of Civil Engineering, and the United States Environmental Protection Agency laboratory in Lexington, MA. The United States Protection Agency's Environmental Research Laboratory in Narragansett, RI was asked to compile and report on the results.

Samples were collected during both wet and dry weather conditions. Point-source sampling sites included two sewage treatment plants and associated bypasses and two combined sewer overflows (CSO). Water samples were also collected from five tributary rivers and from six locations in the Providence River. Samples collected for the wet weather portion of the study were divided into pre-, mid-, and post-storm components.

Marine toxicity tests were conducted with the macroalga Champia parvula, and the sea urchin Arbacia parvula. Freshwater tests were performed using Ceriodaphnia dubia.

The results suggest a general reduction of Providence River water quality relative to the lower bay. There appeared to be a spatial toxicity pattern with toxicity decreasing with increasing distance from the Field's Point discharge. There was no consistent indication that the water quality was affected by storm events.

The results of this study give a very preliminary, short-term image of water quality conditions in the Providence River during the summer of 1989 and some suggestion of possible cause for those conditions. It was determined that the various point source discharges are contributing toxic materials to the river. What is not immediately evident, nor was it within the scope of this study, is the identification of the toxic agent(s) of each discharge and to what extent the observed deterioration of river water quality is due to these discharges.

NARRAGANSETT BAY PROJECT

REPORT ON THE PROVIDENCE RIVER TOXICITY TESTING WET/DRY STUDY

INTRODUCTION

The Narragansett Bay Project has, as one of its primary concerns, the protection of the Bay's ecosystem and living marine resources. The Project's review of previous studies has indicated that pollution-related biological and water quality impacts are largely confined to the Providence River and upper Narragansett Bay. Lacking, however, was the identification of specific toxic point and non point-sources necessary for the Project to recommend pollution control or abatement measures. Consequently, the Narragansett Bay Project contracted with the University of Rhode Island Department of Civil Engineering to collect samples, the Narragansett Bay Commission (Cosper Environmental Services, Inc., contractor) to conduct saltwater toxicity tests, and the U.S. Environmental Protection Agency's Region I Laboratory in Lexington, MA to collect samples and conduct freshwater toxicity tests for the purpose of evaluating the relative toxicity of freshwater inputs to the Providence

River during the summer of 1989. The United States Environmental Protection Agency's Environmental Research Laboratory in Narragansett, RI (ERL-N) was asked to compile and prepare a data report on the results of the toxicity tests. Because of miscommunications, ERL-N did, in fact, conduct some toxicity tests on one series of saltwater samples.

The freshwater inputs studied in this project (Figure 1) included five tributary rivers (Blackstone, Ten Mile, Moshassuck, Woonasquatucket, and Pawtuxet), the Field's Point (FP) and Blackstone Valley District Commission (BVDC) sewage treatment plants and bypasses, and two combined sewer overflows. The sewage treatment plant primary discharges were chlorinated effluents. The effects of effluent chlorination on Providence River water was examined in a separate study (Petrocelli, 1989). In addition, water samples from six stations in the Providence River were tested.

The study was conducted in both wet and dry weather periods to evaluate the impact of the combined sewer overflows (CSO) and sewage treatment plant (STP) bypasses during storm events. The primary goal of this study was to qualitatively evaluate the toxic impact of these freshwater sources to the Providence River system. Partitioning the study into wet and dry weather components begins the process of identifying specific sources of toxicity. Results of these toxicity tests will be incorporated into the Narragansett Bay Project's data base of information concerning protection of public health, living marine resources, and the Bay's ecosystem.

Other possible sources of significant pollution to the Providence River such as non point-source runoff and contaminated sediments were not considered in this study.

METHODS

Sample collections were conducted by personnel from the University of Rhode Island and EPA's Lexington laboratory. Three sets of samples were collected: the wet-weather samples on May 10-11 and June 13, 1989, and the dry-weather samples on August 2, 1989. Locations of all sampling sites are shown in Figure 1.

The toxicity tests employed in this study are part of a series of procedures that have been in used in the United States for, among other things, development of regulatory permits for effluent discharges. The three toxicity test procedures used in this study utilize survival and/or reproductive responses of a marine macroalga, Champia parvula, the sea urchin, Arbacia punctulata, (USEPA, 1988) and a cladoceran, Ceriodaphnia dubia (USEPA, 1985).

Samples from the five tributary rivers were tested using the cladoceran, Ceriodaphnia dubia, survival and reproduction test by EPA's Region I's Lexington laboratory. Effluents from the two sewage treatment plants and associated bypasses, the two combined storm overflows, and the seven Providence River stations were tested by Cosper Environmental Services, Inc. and ERL-N (Table 1) using the marine macroalga Champia parvula and sea urchin Arbacia punctulata procedures. The following is a brief description of the toxicity tests used in this study.

Ceriodaphnia dubia

This test is designed to measure the chronic toxicity of whole effluents and ambient freshwaters. Ceriodaphnia neonates were exposed to a sample in a daily renewal system until 60% of the surviving control organisms produced three broods of offspring (seven days). The endpoints of the test were based on statistically significant adverse effects observed in survival and reproduction. Control water was from Medway Pond, a clean water source approximately 32 miles southwest of the Lexington laboratory.

Champia parvula

This toxicity test uses the sexual phase in the life cycle of this macroalga. Male and female plants were exposed to the STP effluent or Providence River water for two days. The female plants then were transferred to control seawater for a 5- to 7-day recovery period. The recovery period allowed time for cystocarps (evidence of sexual reproduction) to develop. Reduction in the numbers of cystocarps relative to the control was evidence of toxicity. Control water was from the West Passage of Narragansett Bay.

Arbacia punctulata

This sea urchin sperm cell test is a modification of a procedure developed by Dinnel, et. al (1983). Dilute solutions of sea urchin sperm were exposed to the STP effluent or

Providence River water for one hour. Eggs were added following the exposure period and fertilization occurred in the exposure vials. Toxicity was indicated by a reduction in the number of eggs fertilized in relation to the control. Control water was from the West Passage of Narragansett Bay.

Statistical Analysis

The statistical procedures were those prescribed in the marine and freshwater short-term chronic toxicity test manuals (USEPA, 1985, 1988). Basically all test results were first compared to controls using a one-way analysis of variance. If differences were evident, analyses were performed to identify which specific treatments were different from the controls. The Ceriodaphnia mortality data were analyzed by the Lexington laboratory with Fisher's Exact Test (Fisher, 1973) and reproduction data with Dunnett's or Steel's Many-one Rank (Steel, 1959) tests. Champia and Arbacia effluent test results were analyzed with Dunnett's Procedure (Dunnett, 1955) to determine No Observed Effect (NOEC) and Lowest Observed Effect (LOEC) Concentrations. Toxicity of Providence River samples were compared to toxicity reference water collected from lower Narragansett Bay by one-sided t-tests.

RESULTS

Effluent test results (Tables 2 & 3) are presented as short-term chronic values (geometric mean of each pair of NOEC and LOEC values). The chronic value is a standard point estimate of a

presumably 'safe' concentration, lying between the NOEC and LOEC. Results of tributary and Providence River tests are shown as percent of the control response (Tables 4 & 5). Specifics of each series of toxicity tests are included in the respective reports from the Lexington and Cosper laboratories (Appendix A and B respectively).

Effluent Sources

Sewage treatment plant effluent toxicity ranged from less than 0.6% to greater than 10.0 % effluent (Tables 2 & 3). Three of the effluent tests were toxic at the lowest concentration tested and four indicated no toxicity at the highest concentration tested thereby precluding calculation of chronic values. These tests are indicated by a 'less-than' (<) or 'greater-than' (>) symbol in Tables 2 and 3. The available STP data are not sufficient to allow definitive determination of differences of effluent toxicity between wet and dry conditions. Toxicity of CSOs, tested only during storm events, ranged from 6.0% to 12.7% (Tables 2 & 3).

Freshwater Sources

None of the samples from the five tributary rivers caused any significant cladoceran mortality (Table 4). One sample from the Moshassuck River (post-storm) collected May 11 did affect reproduction of *C. dubia*.

Receiving Water Effects

All of the Providence River samples were toxic to C. parvula (Tables 5 & 6). Samples collected on June 13 (Table 6) also caused significant plant mortality in addition to reducing cystocarp production. The sea urchin tests indicated toxicity only in samples collected near the Field's Point discharge (boil).

It should be noted that the results of C. parvula tests on samples collected May 10 & 11 are not presented because control treatments did not meet the minimum criterion for cystocarp production. Also, because of communication and scheduling problems, the effluents collected on June 13 were tested at only two concentrations.

SUMMARY/CONCLUSIONS

Based on the results of this study several preliminary conclusions can be reached:

1. There is a deterioration of water quality in the Providence River relative to lower Narragansett Bay. River waters in the vicinity of the Field's Point discharge are the most severely affected with toxicity tending to decrease with increasing distance (both up and down-stream) from the Field's Point discharge.

2. Results of the Ceriodaphnia dubia tests suggest only minimal toxicity associated with any of the five tributaries included in

this study. However, there is no way to compare the relative sensitivities of the three test species to different complex mixtures such as the tributary and Providence River waters. Therefore, it cannot be conclusively stated that the Champia response to Providence River water, for example, was not due in some part to tributary inputs.

3. Effluent sources to the Providence River, both sewage treatment plant and combined sewer overflows, discharge toxic materials. What is not available, however, is knowledge of the volumes of these materials or their distribution and dilution in the river. This effectively precludes relating a cause and effect between the observed effluent and receiving water toxicities.

4. The limited data from this study do not allow definitive clarification of the significance of wet versus dry weather conditions on the water quality of the river.

This study has attempted to discriminate between the various sources of toxicity to the Providence River. The conclusions drawn are valid but are only preliminary. Much more work needs to be performed before any definitive answers can be given to the questions concerning the sources of degradation to the Providence River. More information on the toxicity and magnitude of all inputs (e.g., effluents, non-point source runoff, sediments, etc.) to the Providence River, coupled with the hydrography of

the river, must be determined to allow development of a 'toxicity budget'.

REFERENCES

Dinnel, P., Q. Stober, J. Link, M. Letourneau, W. Roberts, S. Felton, and R. Nakatani. 1983. Methodology and Validation of a Sperm Cell Toxicity Test for Testing Toxic Substances in Marine Waters. Final Report. Grant R/TOX. FRI-UW-83. University of Washington Sea Grant Program in cooperation with the U.S. Environmental Protection Agency. 208 pp.

Dunnett, C.W. 1955. Multiple Comparison Procedure for Comparing Several Treatments with a Control. Journal American Statistical Association. 50:1096-1121.

Fisher, R.A. 1973. Statistical Methods for Research Workers. 14th edition. Hafner. NY, NY.

Petrocelli, E.A., et. al. 1989. Effects of Chlorine on the Toxicity of a Waste Water Treatment Facility and Impacts on Receiving Waters. Internal Report. ERL-N Contribution # .

Steel, R.G. 1959. A Multiple Comparison Rank Sum Test: Treatments versus Control. Biometrics 15:560-572.

US EPA. 1985. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms. EPA/600/4-85/014.

US EPA. 1988. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Marine and Estuarine Organisms. EPA/600/4-87/028.

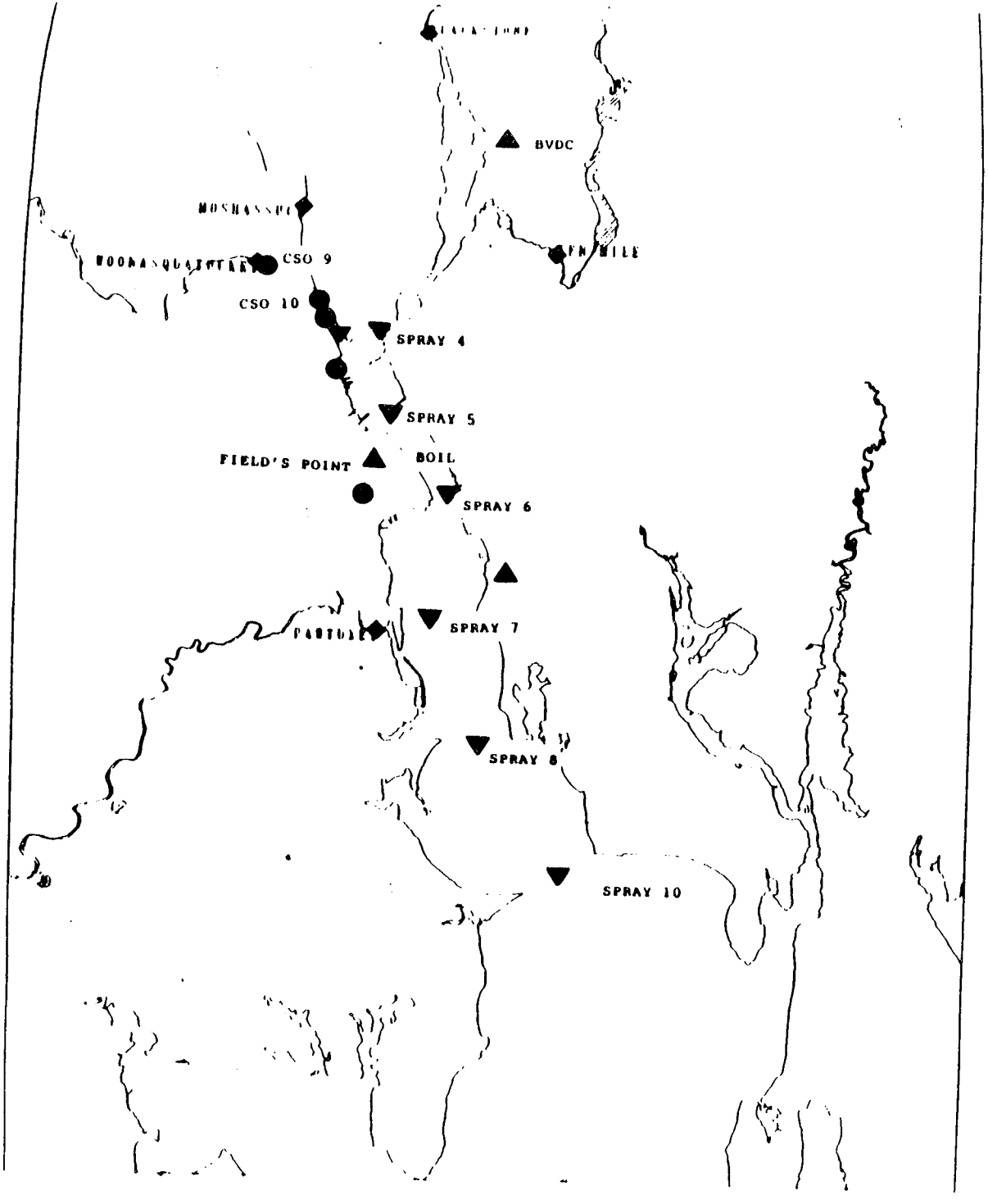

Figure 1.

Wet Weather Study Station Locations

- ▼ Providence River Stations
- ◆ Tributary Stations
- ▲ Sewage Treatment Plants
- ● Combined Sewage Overflow

Table 1. Toxicity tests conducted by C = Cosper Environmental Services, L = US EPA Lexington Laboratory, and E = ERL Narragansett.

STATION	MAY WET WEATHER			JUNE WET WEATHER			DRY WEATHER
	PRE STORM	STORM	POST STORM	PRE STORM	STORM	POST STORM	
STPs							
FIELD'S POINT	C	C	C	-	E	-	C
FIELD'S POINT BYPASS	-	C	-	-	E	-	-
BVDC	C	C	C	-	E	-	C
BVDC BYPASS	-	C	-	-	E	-	-
CSOs							
CSO 9	-	C	-	-	-	-	-
CSO 10	-	C	-	-	E	-	-
PROVIDENCE RIVER							
FIELD'S POINT BOIL	C	C	C	-	E	-	-
SPRAY 4	C	C	C	-	E	-	C
SPRAY 5	C	C	C	-	E	-	C
SPRAY 6	C	C	C	-	E	-	C
SPRAY 7	-	-	-	-	-	-	C
SPRAY 8	C	C	C	-	E	-	C
SPRAY 10	-	-	-	-	-	-	C
TRIBUTARY RIVERS							
MOSHASSUCK	L	L	L	-	-	-	L
BLACKSTONE	L	L	L	-	-	-	L
TEN MILE	L	L	L	-	-	-	L
PAWTUXET	L	L	L	-	-	-	L
WOONASQUATUCKET	L	L	L	-	-	-	L

- Test not performed.

Table 2. Results of effluent toxicity tests conducted by Cosper Environmental Services, Inc. Wet weather samples were collected May 10 and dry weather samples August 2, 1989. Data are presented as short-term chronic values.

STATION	CHRONIC VALUE (% effluent)			
	WEATHER CONDITIONS			
	C. parvula		*A. punctulata*	
	WET	DRY	WET	DRY
SEWAGE TREATMENT PLANTS				
FIELD'S POINT	*	3.1	6.0	6.7
FIELD'S POINT BY-PASS	*	---	2.8	---
BVDC	*	< 0.6	2.8	6.0
BVDC BY-PASS	*	---	< 2.0	---
COMBINED SEWER OVERFLOWS				
CSO 9	*	---	6.0	---
CSO 10	*	---	12.7	---

* Test did not meet minimum criterion for control response.
--- Test was not conducted.

Table 3. Results of wet weather toxicity tests conducted by ERL-N on effluent samples collected June 13, 1989. Data are presented as short-term chronic values. Test concentrations were 5 & 10% for Champia parvula and 10 & 70% for Arbacia punctulata.

STATION	CHRONIC VALUE (% Effluent) WET WEATHER COLLECTION	
	C. parvula	A. punctulata
SEWAGE TREATMENT PLANTS		
FIELD'S POINT	7.1 *	> 10.0
FIELD'S POINT BYPASS	7.1 *	> 10.0
BVDC	7.1 **	> 10.0
BVDC BY-PASS	< 5.0 **	> 10.0
COMBINED SEWER OVERFLOWS		
CSO 10-201	7.1 *	> 10.0
CSO 10-202	> 10.0 *	> 10.0

* Necrotic tissue evident at 5 % effluent concentration.
** Necrotic tissue evident at 10 % effluent concentration.

Table 4. Results of Ceriodaphnia dubia toxicity tests conducted EPA Lexington on tributary samples. Survival and reproductive data are presented as percent of control. Pre-storm samples were collected just before initial rainfall, peak at the height of storm, and post at cessation of storm.

RIVER	% SURVIVAL (7-DAY)	% REPRODUCTION
WET WEATHER SAMPLES (MAY 10, 1989)		
PAWTUXET		
PRE-STORM	88.9	62.6
PEAK	100.0	56.6
POST-STORM	111.1	84.3
BLACKSTONE		
PRE-STORM	111.1	92.5
PEAK	111.1	84.7
POST-STORM	111.1	96.4
MOSHASSUCK		
PRE-STORM	111.1	84.3
PEAK	77.8	61.9
POST-STORM	88.9	36.7 *
TEN MILE		
PRE-STORM	100.0	66.5
PEAK	111.1	87.2
POST-STORM	111.1	78.6
WOONASQUATUCKET		
PRE-STORM	111.1	69.8
PEAK	88.9	66.9
POST-STORM	100.0	81.1
DRY WEATHER SAMPLES (AUGUST 2, 1989)		
PAWTUXET	100.0	114.1
BLACKSTONE	90.0	117.3
MOSHASSUCK	100.0	85.5
TEN MILE	90.0	95.6
WOONASQUATUCKET	90.0	107.6

* Statistically significant reduction from control ($\alpha = 0.05$).

Table 5. Results of Providence River receiving water toxicity tests of May 10, 1989 conducted by Cosper Environmental Services, Inc. Sample concentration represents the percentage of ambient water in the test solutions. Pre-storm samples were collected just before initial rainfall, by-pass samples at initiation of STP by-pass flows, and peak samples at the height of the storm event.

STATION	*C. parvula*		*A. punctulata*	
	sample conc.	cystocarps (% of control)	sample conc.	fertilization (% of control)
WET WEATHER SAMPLES				
SPRAY 4				
PRE-STORM	50	*	80	133
BY-PASS	50	*	73	100
PEAK	50	*	74	122
SPRAY 5				
PRE-STORM	50	*	79	92
BY-PASS	50	*	81	65
PEAK	50	*	77	115
SPRAY 6				
PRE-STORM	50	*	78	113
BY-PASS	50	*	79	118
PEAK	50	*	82	83
F.P. BOIL				
PRE-STORM	50	*	81	3 **
BY-PASS	50	*	81	0 **
PEAK	50	*	82	2 **
DRY WEATHER SAMPLES				
SPRAY 4	50	56 **	93	100
SPRAY 5	50	52 **	93	91
SPRAY 6	50	29 **	92	102
SPRAY 7	50	46 **	92	96
SPRAY 8	50	83 **	95	100
SPRAY 10	50	83 **	100	100

* Test did not meet minimum criteria for control response.
** Statistically significant reduction from control ($\alpha = 0.05$).

Table 6. Results of wet weather toxicity tests of Providence River samples. Tests were conducted by staff of ERL-N on samples collected June 13, 1989. Data are presented as percentage of control.

STATION	*C. parvula* (% cystocarp)	*A. punctulata* (% fertilization)
SPRAY 4	a	101.7
SPRAY 5	a	104.2
SPRAY 6	a	101.4
SPRAY 8	46.0 *	100.0
BOIL C	a	8.3 *
BOIL D	0.0 b *	4.2 *

a Plants killed.
b Plants stunted.
* Statistically significant reduction from control ($\alpha = 0.05$).

APPENDIX A

REPORT OF TOXICITY TESTS ON PROVIDENCE RIVER TRIBUTARIES

Conducted by

US EPA LEXINGTON, MA

Narragansett Bay
Wet Weather Study
Toxicity Tests of Tributaries

On May 10, 1989, three samples for each of five tributaries of Narragansett Bay were delivered to EPA Region I Lab for toxicity testing. The tributaries sampled were the Pawtuxet, Blackstone, Mosshassuck, Ten Mile and Woonasquatucket Rivers. The three samples of each were referred to as Run A, preceding the rain event; Peak (of the rain event); and Run C, after peak of the storm.

The test organism employed was Ceriodaphnia dubia. The test methods used are those specified in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/600/4-85/014).

The test was concluded on May 17 after 80% of the controls produced a third brood. Medway Pond water was used as laboratory control water.

Fisher's Exact Test was used to determine whether mortality in the Peak and Run C were significantly different than in the pre-storm sample. Dunnett's test was used to determine whether a significant difference existed between reproduction in the pre-storm sample and the peak and Run C samples.

All results are summarized in Table 1. The only significant effect observed was in the Mosshassuck River. The Run C sample produced significantly less young than the prestorm (Run A) sample.

Narragansett Bay Wet Weather Study
Ceriodaphnia dubia
Chronic Toxicity Test
May 10, 1989

Pawtuxet River

Sample	Mean No. Young/Female	Percent Survival Day 3	Day 5	Day 7
Run A	17.6	100	80	80
Peak	15.9	90	90	90
Run C	23.7	100	100	100

Blackstone River

Run A	26	100	100	100
Peak	23.8	100	100	100
Run C	27.1	100	100	100

Mosshassuck River

Run A	23.7	100	100	100
Peak	17.4	100	80	70
Run C	10.3 *	100	80	80

Ten Mile River

Run A	18.7	100	90	90
Peak	24.5	100	100	100
Run C	22.1	100	100	100

Woonasquatucket River

Run A	19.6	100	100	100
Peak	18.8	80	80	80
Run C	22.8	100	90	90

Control--Medway Pond Water				
Control	28.1	100	100	90

*significantly different than control based on Fisher and Dunnett's tests for survival and reproduction, respectively.

TEST: WET WEATHER - PAWTUXET R.
DATE START: MAY 10, 1989 Start time 1630 PAGE 1

EXPOSURE	Day	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
RUN A	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	pairs
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EFJ
	4	✓	✓ 3	✓ 3	✓ 4	✓ 3	✓ 5	✓ 2	✓ 3	D	D 5	EFJ
	5	✓ 3	✓ 7	✓ 6	✓ 7	✓ 7	✓ 7	✓ 9	✓ 10			EFJ
	6	✓ 8	✓ 1	✓ 12	✓ Ø	✓ 11	✓ 9	✓ 12	✓ 13			EFJ
	7	✓ 14	✓ 11	✓	✓ 8	✓	✓	✓	✓	↓	↓	EFJ
		3/25	3/19	3/21	3/19	3/21	3/19	3/23	3/26	D	1/3	$\bar{x}$ = 1960
PEAK	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	pairs
	3	✓	✓	✓	✓	✓	✓	D	✓	✓	✓	EFJ
	4	✓	✓ 4	✓ 5	✓ 3	✓	✓ 4	↓	✓ 3	✓	✓	EFJ
	5	✓ 3	✓ 2	✓ 7	✓ 10	✓ 4	✓ 6		✓ 6	✓	✓	EFJ
	6	✓ 9	✓	✓ 15	✓	✓ 8	✓		✓	✓	✓	EFJ
	7	✓	✓ 17	✓	✓ 9	✓ 14	✓ 13	↓	✓ 16	✓	✓	EFJ
		2/12	3/23	3/27	3/23	3/26	3/23	D	3/25	0/0	0/0	15.9
RUN C	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	pairs
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EFJ
	4	✓ 4	✓ 4	✓ 4	✓ 4	✓ 3	✓ 4	✓ 5	✓ 4	✓	✓ 3	EFJ
	5	✓ 10	✓ 8	✓ 9	✓	✓ 5	✓ 7	✓ 8	✓ 5	✓ 5	✓ 10	EFJ
	6	✓	✓ 8	✓ 15	✓ 6	✓ 9	✓	✓	✓ 15	✓ 11	✓ 12	EFJ
	7	✓ 14	✓	✓ 1	✓	✓	✓ 14	✓ 15	✓ 17	✓ 15	✓	EFJ
		3/28	3/20	3/29	2/10	3/17	3/25	3/28	4/41	3/31	3/20	$\bar{x}$ = 28.9
									3/24			

✓=Alive
D=Dead Adult

#= Number of young/adult

TEST: [illegible]

DATE START: [illegible]

EXPOSURE	Day.	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
[illegible] P	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2											
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	4	✓/3	✓/3	✓/4	✓	✓/5	✓/4	✓/5	✓/2	✓/1	✓/3	mDB
	5	✓/10	✓/6	✓/7	✓/5	✓/8	✓/7	✓	✓/4	✓/9	✓	mDB
	6	✓/12	✓/11	✓/14	✓/12	✓	✓	✓/11	✓/13	✓/14	✓/8	EAJ
[illegible] 6.0	7	✓	✓	✓	✓/14	✓/8	✓/13	✓/18	✓/12	✓/10	✓/16	EAJ
		3/[illegible]	3/26	3/25	3/[illegible]	3/21	3/24	3/34	4/31 3/19	3/24	3/27	
PC-7K	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	mDB
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	mDB
	4	✓/4	✓/5	✓/4	✓/4	✓/4	✓/4	✓/2	✓/4	✓/4	✓/1	mD
	5	D/5	✓	✓/7	✓/8	✓/8	✓/9	✓/7	✓/8	✓/7	✓/5	EAJ
	6	✓	✓/8	✓/13	✓/12	✓/11	✓/12	✓/14	✓/15	✓/9	✓	EAJ
	7	✓/13	✓/17	✓	✓	✓	✓/1	✓	✓	✓	✓/13	EAJ
[illegible] B		✓ [illegible]	3/[illegible]	3/24	3/[illegible]	3/23	4/26 4/26	3/23	3/27	3/20	3/19	
B.N.C.												
	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	mD
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	mDB
	4	✓/5	✓/4	✓/4	✓/4	✓/4	✓/3	✓/4	✓/4	✓/5	✓/4	mDB
	5	✓/7	✓/5	✓/9	✓/11	✓/7	✓/9	✓/11	✓/10	✓/11	✓/9	EAJ
	6	✓/15	✓/13	✓/16	✓/13	✓/12	✓	✓/14	✓/17	✓/16	✓/13	EAJ
	7	✓	✓/5	✓	✓	✓	✓/14	✓	✓	✓	✓	EAJ
X = 27.1		3/27	4/27 4/22	3/29	3/[illegible]	3/23	3/26	3/29	3/31	3/30	3/26	

✓=Alive
D=Dead Adult

#= Number of young/adult

TEST: [redacted]

DATE START: [redacted]

PAGE

EXPOSURE	Day	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
[redacted]	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	PMM
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EAJ
	4	✓	✓/2	✓/3	✓/3	✓/4	✓/5	✓/4	✓/3	✓/4	✓/4	EAJ
	5	✓/8	✓/10	✓/10	✓/8	✓/10	✓/9	✓/8	✓/8	✓/7	✓/10	EAJ
	6	✓/15	✓/12	✓/13	✓/13	✓	✓/11	✓	✓/10	✓	✓/12	EAJ
	7	✓	✓	✓	✓	✓/8	✓	✓/12	✓	✓/11	✓	EAJ
		[redacted]	3/31	3/27	3/24	3/22	3/25	3/27	3/21	3/19	3/26	
PC+K	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	PMM
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EAJ
	4	✓	✓/2	✓/3	✓/4	✓/3	✓/4	✓/4	✓/4	✓/4	✓/3	EAJ
	5	✓	D	✓/8	D	✓/9	✓/7	✓/9	✓/7	✓/11	✓/9	#lost count/D's in between
	6	✓	↓	✓	↓	✓	✓	✓	D	✓/14	✓/11	EAJ
	7	✓	↓	✓/11	↓	✓/18	✓/12	✓/16	↓	✓	✓/1	EAJ
- 17.4 [redacted]		[redacted]		3/22	3/17	3/30	3/23	3/29	2/11	3/29	3/24	
(*) RUN C	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	PMM
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EAJ
	4	✓/3	D	✓/4	✓/4	✓	D/4	✓/3	✓/2	✓	✓/4	EAJ
	5	✓/7	↓	✓/4	✓	✓	↓	✓/6	✓/5	✓	✓/6	EAJ
	6	✓/13	↓	✓	✓	✓	↓	✓	✓/11	✓	✓/1	EAJ
	7	✓/1	↓	✓/7	✓/5	✓	↓	✓/6	✓	✓	✓/7	EAJ
[redacted]		3/24	0/0	3/15	2/9	0/0	1/4	3/15	3/18	0/0	3/18	
103												

✓=Alive
D=Dead Adult

#= Number of young/adult

TEST: [illegible]

DATE START: [illegible]

PAGE [illegible]

EXPOSURE	Day	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
[illegible]	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	—	—	—	—	—	—	—	—	—	—	[illegible]
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDR
	4	✓/1	✓/2	✓/3	✓/4	✓/4	✓/4	✓/4	D/4	✓/4	✓	MDB
	5	✓ 8	✓ 9	✓ 5	✓ 1	✓ 8	✓ 8	✓ 7	↓	✓ 6	✓ 6	EDB
	6	✓ 12	✓ 7	✓ 13	✓ 14	✓	✓ 6	✓ 10	↓	✓ 13	✓ 8	EDB
	7	✓/1	✓/2	✓/1	✓/[illegible]	✓ 15	✓ 13	✓	↓	✓ 12	✓ 13	MDB/EDB
137		3/ 2	3/ 8	3/ 1	3/20	3/27	3/26	3/21	1/4	3/23	3/27	
Check	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[illegible]
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	4	✓/3	✓/4	✓/4	✓/4	✓/3	✓/4	✓/3	✓/3	✓/4	✓/4	MDB
	5	✓ 9	✓ 10	✓ 7	✓ 9	✓ 7	✓ 8	✓ 10	✓ 11	✓ 9	✓ 8	EDB
	6	✓ 11	✓ 12	✓ 12	✓ 11	✓	✓	✓ 14	✓	✓ 15	✓ 8	EDB
	7	✓	✓ 13	✓ 16	✓ 16	✓ 14	✓ 11	✓	✓ 13	✓	✓ 11	EDB
		3/[illegible]	3/[illegible]	3/[illegible]	3/[illegible]	3/24	3/23	3/27	3/27	3/25	3/20	
RUNC												
	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	[illegible]
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	4	✓/3	✓/3	✓/4	✓/3	✓/2	✓/3	✓/3	✓	✓/4	✓/4	MDB
	5	✓ 9	✓ 8	✓ 5	✓ 7	✓ 10	✓ 8	✓ 9	✓	✓ 8	✓ 8	EDB
	6	✓ 12	✓ 10	✓ 14	✓ 13	✓	✓ 15	✓ 15	✓	✓ 14	✓ 13	EDB
	7	✓	✓	✓	✓ 14	✓ 14	✓	✓	✓	✓	✓	EDB
$\bar{x}$ = 22.1		3/[illegible]	3/21	3/23	3/23	3/26	3/26	3/27	0/0	3/26	3/25	

✓=Alive
D=Dead Adult

#= Number of young/adult

TEST:
DATE START:

EXPOSURE	Day	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
[illegible]	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MPB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MPB
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EAJ
	4	✓ 7	✓ 4	✓ 3	✓ 3	✓ 3	✓ 5	✓ 2	✓ 3	✓ 1	✓ 3	EAJ
	5	✓ 6	✓ 4	✓ 9	✓ 7	✓ 7	✓ 6	✓ 8	✓ 8	✓ 7	✓ 9	EAJ
	6	✓ 11	✓ 7	✓	✓	✓	✓ 11	✓ 7	✓	✓ 9	✓ 11	EAJ
	7	✓	✓	✓ 9	✓ 6	✓ 12	✓ 1	✓	✓ 9	✓	✓	EAJ
$\bar{X}$ = 9.6		3/21	3/15	3/21	3/16	3/22	3/22	3/18	3/20	3/17	3/23	
Duck	1	✓	✓	✓	✓	✓	✓	✓	✓	D	✓	MPB
	2	✓	✓	✓	✓	✓	✓	✓	✓		✓	MPB
	3	✓	✓	✓	✓	✓	✓	✓	D		✓	EAJ
	4	✓ 4	✓ 4	✓ 4	✓ 5	✓ 4	✓ 4	✓ 3			✓ 3	EAJ
	5	✓ 8	✓ 8	✓ 7	✓ 10	✓ 8	✓ 7	✓ 7			✓ 6	EAJ
	6	✓ 13	✓ 10	✓ 11	✓ 12	✓ 12	✓ 13	✓ 13			✓	EAJ
	7	✓ 7	✓	✓ 1	✓ 13	✓	✓	✓			✓ 13	EAJ
$\bar{X}$ = 18.8		3/25	3/22	3/22	3/25	3/24	3/24	3/23	D/D	9/6	3/22	
RUNC												
	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MPB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MPB
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	EAJ
	4	D 4	✓ 3	✓ 4	✓ 5	✓ 2	✓ 2	✓ 2	✓ 3	✓ 2	✓ 3	EAJ
	5		✓ 5	✓ 10	✓ 9	✓ 9	✓ 11	✓ 5	✓ 7	✓ 8	✓ 8	EAJ
	6		✓ 13	✓ 14	✓ 14	✓ 14	✓ 14	✓ 9	✓ 11	✓ 12	✓ 15	EAJ
	7	✓	✓ 16	✓ 1	✓	✓ 16	✓	✓ 15	✓ 13	✓	✓	EAJ
$\bar{X}$ = 22.8		1/4	3/21	3/21	3/28	3/25	3/27	3/22	3/14	3/22	3/26	

✓=Alive
D=Dead Adult

#= Number of young/adult

TEST:
DATE START:

PAGE 6

EXPOSURE	Day	ANIMAL NUMBER 1	2	3	4	5	6	7	8	9	10	Remarks
[illegible]	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2										✓	
	3			✓		✓		✓		✓	✓	MDB
	4			✓/4		✓/4		✓		✓/2	✓	MDB
	5			✓/4		✓/6		✓/3		✓/5	✓/4	EFJ
	6			✓		✓/7		✓/7		✓	✓/8	EFJ
	7	↓	↓	✓/5	↓	✓	↓	✓	↓	✓/3	✓/8	MDB
$\bar{X}$ = 7.0		✓/0	✓/0	3/13	✓/0	3/17	✓/0	2/10	✓/0	3/10	3/20	
FISH TANK	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	4	✓/4	✓/1	✓	✓	✓/3	✓	✓/1	✓	✓	✓/2	MDB
	5	✓	✓/6	✓/3	✓	✓/1	✓	✓	✓	✓	✓	EFJ
	6	✓	✓	✓/1	✓	✓/1	✓	✓/6	✓	✓/4	✓/7	EFJ
	7	✓	✓/7	✓	✓	✓	✓/9	✓	✓/2	✓	✓	MDB
$\bar{X}$ 5.8		1/4	3/14	2/4	0/0	3/5	1/9	2/7	1/2	1/4	2/9	
Mentally Ill	1	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MB
	2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	MDB
	3	✓	✓	✓	✓	✓	✓	✓	✓	✓/4	✓	MB
	4	✓/3	✓/4	✓	✓/6	✓/4	✓/6	✓/2	✓/7	✓/8	✓/4	MDB
	5	✓/10	✓/11	✓/4	✓/14	✓/11	✓/12	✓/12	✓/9	✓	✓/11	*SCHIZOAFFECTIVE, MANIC
	6	✓/16	D	✓/11	✓/18	✓/17	✓	✓/15	✓/15	✓/17	✓/16	EFJ
	7	✓	↓	✓	✓	✓	✓/17	✓	✓	✓/15	✓	MDB
$\bar{X}$ = 28.1		3/29	2/15	2/15	3/38	3/32	3/35	3/29	3/28	4/44	3/31	
										3/29		

✓=Alive
D=Dead Adult

#= Number of young/adult

NARRAGANSETT BAY & PROVIDENCE RIVER
DRY WEATHER TOXICITY TESTING

A component of the Narragansett Bay Wet Weather Study entails chronic toxicity testing of samples from five tributaries, six stations in the Providence River and Narragansett Bay and two effluents of wastewater treatment plants, during a period of dry weather.

Sampling for this work was conducted August 2, 1989 by Region I EPA Laboratory biologists and Dr Raymond Wright of the University of Rhode Island, Department of Civil Engineering. Grab samples were collected at three to four hour intervals over a period of twelve hours, in the five major tributaries of the Providence River: the Mosshasuck, Blackstone, Ten Mile, Pawtuxet, and Woonasquatucket Rivers. Th staions sampled were the same as those sampled during the wet weather event.

Postchlorinated samples were also collected from Field's Point WWTP and Blackstone Valley District Commission (BVDC) WWTP.

Stations were also sampled in the Providence River and Narragansett Bay at three to four hour intervals (Figures 1-2).

All samples were iced. The samples from Field's Point, BVDC, and the Providence River were picked up by George Morrisson of EPA ERL-Narragansett for shipment to Cosper Laboratory. Cosper will perform chronic toxicity tests using the sea urchin, Arbacia punctulata and the macroalga, Champia parvula.

The tributary samples were transported to the EPA New England Regional Laboratory for chronic toxicity testing. The test method employed is the Cladoceran Survival and Reproduction Test in Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms (EPA/600/4-89/001). Samples from each tributary were composited. Initial test chemistry is shown in table 2.

Survival was checked in each test daily and samples were renewed in each test vessel daily.

RESULTS

The data from each test are shown in table 1. Fisher's Exact Test was performed on the data for each tributary. No effects were produced on survival. Dunnett's test showed that no significant effects occurred in reproduction in any tributary sample.

Figure 1. Sampling stations in Providence River

Figure 2. Sampling stations (continued)

Site tested: Tributaries of Narragansett Bay
Location: Rhode Island
Type test: Survival and Reproduction Test
Organism: Ceriodaphnia dubia
Test start Date: August 3, 1989
Time start: 0915
No. Replicates: 10
No. Organisms/Rep: 1
Data Analysis: Fisher's test to analyze mortality data first, transforming survival data to arc sine values. Reproduction data analyzed using Dunnett's or Steel's test.

Concentration	Mean Young Ea Female	Std. Dev.	No. Young 1	2	3	4	5	6	7	8	9	10
Control	24.9	2.56	22	26	25	24	26	27	21	24	24	30
Mosshasuck R.	21.3	3.77	20	23	15	28	18	24	20	19	21	25
Woonasquatucket R.	26.8	8.20	32	28	28	4	32	29	31	27	29	28
Pawtuxet R.	28.4	3.47	29	33	30	26	26	25	22	31	31	31
Blackstone R.	29.2	6.97	30	25	22	31	36	38	27	16	30	37
Ten Mile R.	23.8	8.15	23	24	28	26	25	31	3	29	30	19

*Reproduction significantly different than control based on Dunnett's Test (P = .05)

Concentration	Percent Survival day 3	day 5	day 7
Control	100	100	100
Mosshasuck R.	100	100	100
Woonasquatucket R.	90	90	90
Pawtuxet R.	100	100	100
Blackstone R.	100	90	90
Ten Mile R.	90	90	90

**Survival significantly different than control based on Fisher's Exact test (P = .05)

SAMPLE CHEMISTRY

Tested site: Tributaries of Narragansett Bay
Location: Narragansett Bay Dry Weather Study
Test start Date: August 3, 1989

Sample	Day	D.O. Initial	ph	Temp * C	Conductivity uS/cm (2K)
Wonasquatucket R.	-0-	8.7	6.09	19.2	288
Mosshasuck R.		8.4	6.21	17.9	433
Pawtuxet R.		7.8	5.89	15.3	357
Blackstone R.		7.9	5.73	16.1	280
Ten Mile R.		8.7	6.16	16.9	301

*Vessels are warmed to 25 degrees before test organisms added.

APPENDIX B

REPORT OF PROVIDENCE RIVER TOXICITY TESTS

Conducted by

COSPER ENVIRONMENTAL SERVICES, INC.

FINAL REPORT

on

NARRAGANSETT BAY PROJECT
WET AND DRY WEATHER TOXICITY TESTING
WITH THE SEA URCHIN (*Arbacia punctulata*)
AND
THE RED MACROALGA (*Champia parvula*)

to

Narragansett Bay Project
US Environmental Protection Agency - Region I
J.F.K. Federal Building (WQP-2103)
Boston, Massachusetts 02203

15 September 1989

John W. Williams
COSPER ENVIRONMENTAL SERVICES, INC.
Northport Environmental Research Center
Eaton's Neck Road
P.O. Box 525
Northport, NY 11768

TABLE OF CONTENTS

LIST OF TABLES

FINAL REPORT

on

NARRAGANSETT BAY PROJECT WET AND DRY WEATHER TOXICITY TESTING WITH THE SEA URCHIN (Arbacia punctulata) AND THE RED MACROALGA (Champia parvula)

to

NARRAGANSETT BAY PROJECT
EPA - Region I

15 September 1989

INTRODUCTION

The objective of this study was to characterize the toxicity of discharges from sewage treatment plants (STPs) and other point sources into Narragansett Bay, Rhode Island, and to measure the resulting toxicity of ambient waters during wet and dry weather conditions. A series of pre-storm and rain storm event samples were collected by US Environmental Protection Agency (EPA) personnel and delivered by Cosper Environmental Services, Inc. (CES) to its laboratory complex located in Northport, New York on 11 May 1989. A second series of samples was collected during a dry period on 2 August 1989. These samples were delivered to CES on 3 August 1989. Toxicity of effluent and ambient water samples was measured using two marine species: the sea urchin (Arbacia punctulata) and the red macroalga (Champia parvula). The details of methods used to conduct the toxicity tests, results obtained, and interpretation of results are provided in the following sections of this report.

SAMPLE COLLECTION AND PREPARATION

Wet Weather Study

Twenty-eight one-gallon water samples were collected from river stations, STP post-chlorinated effluents, STP bypass flows, and combined sewer overflows (CSOs) in the upper reaches of Narragansett Bay, Rhode Island during the pre-storm period and the rain event of 10-11 May 1989. Post-chlorinated effluent samples were collected from the Field's Point sewage treatment plant (STP) and the Blackstone Valley District Commission sewage treatment plant (BVDC STP) during the rain event. These sites were also sampled during the time of STP bypass flow (unchlorinated). Two CSOs were sampled, including CSO 9 and CSO 10. Also, five river (ambient water) stations were sampled, including SPRAY 4, SPRAY 5, SPRAY 6, SPRAY 8, and the Field's Point boil. The river station samples included three phases of the storm event: pre-storm samples, samples collected during STP bypass activation, and samples collected during storm event peak flow.

Storm samples were transferred to the custody of CES personnel on 11 May at the Fields Point Treatment Plant, Providence, Rhode Island. Pre-storm samples were picked up at the US Environmental Protection Agency (EPA) Environmental Research Laboratory - Narragansett (ERLN). Samples were stored in cubitainers on ice during transport to CES. Samples arrived at CES at approximately 1430 on 11 May 1989, at which time sample preparation for toxicity tests was initiated immediately.

Samples were characterized for the parameters of temperature, salinity, dissolved oxygen (DO), pH, and total residual chlorine (TRC). Certain samples were composited (Appendix A) according to instructions received from Mr. Steve Schimmel (EPA) and each sample was assigned a CES inventory number. The pre-storm effluent samples collected at Field's Point STP (Run A, post-chlorination) and BVDC STP (Run A, post-chlorination) were not included in the toxicity testing program.

The salinity of each sample was adjusted to 30 o/oo using concentrated seawater brine (102 o/oo) obtained from ERLN. Four of the samples received (FP STP bypass, BVDC STP bypass, CSO 9, And CSO 10) were aerated briefly because DO was observed to be less than 4.0 mg/L in these samples. No further alterations of samples were made. The river station (ambient water) samples (Spray 4, 5, 6, 8, and Field's Point Boil) were tested as whole, salinity-adjusted sample for the sea urchin fertilization tests. For the Champia tests, the salinity-adjusted river samples were further diluted with seawater to obtain a maximum concentration of fifty percent sample. The STP samples (Field's Point and BVDC), the STP bypass samples (Field's Point STP bypass and BVDC STP bypass samples), and the CSO samples (CSO 9 and CSO 10) were run as dilution-series tests which included a minimum of five sample dilutions for each species. Dilution water for the toxicity testing program was Narragansett Bay filtered natural seawater obtained from ERLN. This water was also used as the control solution for each species.

Dry Weather Study

Water samples collected from six river stations and two post-chlorinated STP discharges (Field's Point and BVDC) were collected on 2 August 1989 and transferred to the custody of CES on the same date at ERLN. Samples were stored in cubitainers on ice during transport to CES. Samples arrived at CES at approximately 0800 on 3 August 1989. Preparation of samples for toxicity testing was initiated immediately.

The samples for both the Field's Point STP and the BVDC STP were contained in several cubitainers of various volumes. The Field's Point STP samples were enclosed in six separate containers and the BVDC STP samples were enclosed in four separate containers. The FP and the BVDC samples were composited (Appendix A) according to instructions received by telephone from Mr. Peter Nolan (EPA) and proportionately according to the volume of each container. Each composite sample was assigned a CES inventory number and then was characterized for the parameters of temperature, salinity, DO, pH, and total residual chlorine. The following river stations samples were received: Spray 4 (mouth of Seekonk River, off India Point), Spray 5 (off Sassafras Point), Spray 6 (off Field's Point), Spray 7 (off Sabin's Point), Spray 8 (off Gaspie Point), Spray 10 (off Conimicut Point Light).

The salinity of each sample was adjusted (when necessary) to 30 o/oo using concentrated seawater brine (102 o/oo) obtained from ERLN. No further alterations were made to samples. The river stations were tested as whole, salinity-adjusted sample (for the

sea urchin fertilization tests) or as salinity-adjusted fifty-percent sample (for the Champia parvula sexual reproduction tests). The Field's Point and BVDC STP samples were tested as dilution series tests with a minimum of five sample concentrations. Dilution water for the dry weather study was Narragansett Bay filtered natural seawater obtained from ERLN.

MATERIALS AND METHODS

Sample Characterization

Procedures for characterizing wet and dry weather samples were as follows:

Parameter	Method
Temperature	Glass thermometer
Salinity	Atago refractometer
pH	Orion Research electrode (201, SA201)
Dissolved oxygen	YSI Model 51B DO meter
Total Residual Chlorine	Fisher-Porter Amperometric titration

Instumentation used for sample characterization was calibrated before use.

Test Organisms

Sea Urchin (Arbacia punctulata)

Stocks of fertile male and female sea urchins (Arbacia punctulata) used for the wet weather toxicity tests were obtained on 02 May 1989 from ERLN. A second batch was obtained from ERLN on 11 May 1989 because the yield of gametes from the first batch

had been determined to be poor. Sea urchins for the dry weather study were obtained from Marine Biological Laboratory, Department of Marine Resources on 2 August 1989. Sea urchins were held in a 50-gallon plastic tank with recirculating natural seawater (salinity 28 o/oo) held at a temperature of approximately 15 C. Sea urchins were provided with a supply of fresh brown kelp (Laminaria sp.) throughout the holding period.

Marine Macroalga (Champia parvula)

Branches of mature male and female Champia parvula were received from ERLN in October of 1988. Stock cultures of segregated male and female plants have been maintained in 1-L glass Erlenmeyer flasks at CES since that time. New cultures were routinely started by pinching tips from branches of each sex with dissecting scissors and transferring them to fresh culture medium. Culture medium was prepared with filtered and autoclaved Long Island Sound seawater (28 o/oo salinity). Ten milliliters of nutrient stock (Weber et al., 1988) with disodium ethylenediamine tetraacetate (EDTA) was added per liter of filtered seawater. The algal cultures were held at a temperature of approximately 23 C with a lighting cycle of 16 h light (intensity 300-500 ft-c) and 8 h dark. On the day of test initiation of the wet weather study (11 May 1989) male and female branch tips were removed from the mature cultures and transferred to the test chambers.

For the dry weather study, living male and female plant material was obtained from Dr. Glen Thursby (SAIC/ERLN). These plants, along with exposure and recovery period nutrients, were received on 3

August 1989 along with the test samples.

Test Systems

Sea Urchin (A. punctulata) Fertilization Test

Procedures for the sea urchin fertilization test were based on EPA testing guidelines outlined in Weber et al. (1988) for the east coast sea urchin (Arbacia punctulata). Sea urchin fertilization tests for the wet weather study were initiated on the same day (time, 2346) as the samples arrived at CES. The tests for the dry weather study were also initiated on the same day (time, 1625) as sample receipt. The salinity of the wet weather samples ranged from 0 o/oo to 20 o/oo and the salinity of the dry weather samples ranged from 4 o/oo to 30 o/oo. Salinity was adjusted to 30 o/oo (when necessary) with concentrated seawater brine. This adjustment resulted in dilution of the samples to final sample concentrations ranging from 71 percent to 100 percent (no salinity adjustment) of the original sample. Sea urchin sperm and eggs were exposed under static conditions to salinity-adjusted river station samples and to at least six serial dilutions (0.5 dilution factor) of the STP, STP bypass, and CSO samples.

Approximately 1 h prior to test initiation, spawning was induced in isolated male and female sea urchins by stimulating them with a 10 v electrode. Sperm were collected using a 1-cc syringe as they were released from the gonopores into seawater. Sperm collected from 4 males were pooled in a 20-mL glass scintillation vial and stored on ice. Eggs from 4 females were collected with a 5-cc syringe and transferred to centrifugation tubes. The eggs were

rinsed three times by allowing them to settle in the tubes and replacing the seawater. The eggs were then pooled by transferring them in seawater to a glass jar. They were stored at 20 C.

Sperm were diluted with seawater to obtain a final density of 4.7 (dry weather study) to 9.6 (wet weather study) x 107 cells per mL as determined by sperm cell counts on a Neubauer hemacytometer. Eggs were diluted to 2000 (± 200) eggs per mL with seawater as determined by egg cell counts of a 1-mL aliquot in a counting chamber. The test design included three replicate test vials (20-mL glass scintillation vials) for each treatment during the wet weather study and four replicate vials during the dry weather study. Each vial contained 5 mL of test solution. The solutions were warmed to 20 ± 1 C prior to initiation of the test.

Tests were started by dispensing 100 uL of diluted sperm to each test vial. The test vials were then incubated at 20 C. After 1 h, 1 mL of egg suspension was dispensed to each test vial. The test array was incubated an additional 20 min at 20 C and then the test was terminated by addition of 2 mL of 10 percent formalin to each vial. The total test exposure time was 80 min. Test vials were capped and stored at room temperature until they were opened for egg cell counts. Seawater control treatments (sets of three replicate vials) were randomly positioned within each test array. A reference toxicant test (copper sulfate) was also included within each test array.

A total of 100 eggs from each vial was examined under a compound microscope for the presence or absence of a fertilization

membrane. Eggs that had a fertilization membrane were scored as "fertilized." Those that did not have a fertilization membrane were scored as "unfertilized." Any misshapen or damaged eggs were not included in the tally. An increase in the number of unfertilized eggs, relative to the controls, was the effect measured in this test.

Marine Alga (Champia parvula) Sexual Reproduction Test

Procedures for the algal sexual reproduction test were based upon the EPA guidelines outlined in Weber et al. (1988) for "Algal (Champia parvula) Sexual Reproduction Test." Tests of samples from the Narragansett Bay wet weather event and the dry weather event were initiated on the same day the samples arrived at CES. Male and female branches were exposed to test solutions for two days under static conditions to a 50 percent dilution (salinity adjusted to 30 o/oo) of each of the river station samples. The STP, STP bypass, and the CSO samples were tested as five concentrations of salinity-adjusted sample. The dilution series included concentrations of 0.6, 1.8, 5.4, 16.5, and 50 percent sample. Dilution water controls were included with each dilution-series test and with each group of five river station tests.

Individual female branches were examined under a compound microscope for the presence of trichogynes (reproductive hairs to which spermatia attach) near the apex of branch tips. The male branches were examined for the presence of spermatial sori (thickened areas of the thallus which produce spermatia). Once cultures were determined to be usable for toxicity testing

(trichogynes and sori with spermatia were present), plant cuttings were prepared, using dissecting scissors, or fine-tipped forceps to sever female cuttings (7 to 10 mm) and male cuttings (15 to 20 mm). Female cuttings were prepared first to minimize the chances of contaminating them with water containing spermatia.

Five female cuttings and one male cutting were added to each test chamber. The test chambers were 4-oz. polystyrene disposable beakers containing 100 mL of test solution with three replicate test beakers per concentration (15 female, 3 male branches). The test chambers were capped and placed under cool-white lights set on a 16 h light (475-500 ft-c) and 8 h dark cycle. Each test chamber was supplied with 1 mL of nutrient stock (without EDTA). Test chambers were swirled by hand twice daily. The branches were exposed to test solutions at 23 $\pm$ 1 C for 2 days, during which time the light intensity and temperature were monitored daily.

After the two-day exposure period, the female branches were removed and transferred by replicate (5 branches each) to recovery jars (125-mL glass bottles or flasks) containing 100-mL of recovery medium (including EDTA). The recovery medium was a 1:1 Narragansett Bay seawater and GP-2 mixture. An aeration tube was added to each flask and flasks were aerated for 7 to 9 days under the same lighting and temperature conditions as during the sample exposure period.

At the end of the recovery period, the number of cystocarps per female branch was counted under a stereomicroscope.

Cystocarps are easily distinguished from young branches because they possess an apical opening for spore release (ostiole) and darkly pigmented spores. Reduction in cystocarp production relative to the controls was the effect measured in this test.

Statistical Analysis

Chronic toxicity data from the sea urchin fertilization and the algal sexual reproduction tests were used to determine if the organisms exhibited significant chronic effects when exposed to ambient water, post-chlorinated STP effluent, STP bypass flow, or CSO discharge samples. The sea urchin fertilization data were analyzed using analysis of variance (ANOVA) and Dunnett's multiple range test (if the ANOVA was significant, $P < 0.05$) on arc-sine square root transformed data. The number of unfertilized sea urchin eggs in the test solutions was compared with the number of unfertilized eggs in the control water. The validity of the homogeneity of variance assumption was tested by Bartlett's test. Sample size for the sea urchin fertilization test was 300 eggs per concentration for the wet weather study and 400 eggs per concentration for the dry weather study.

The trimmed Spearman-Karber method with Abbott's correction for unfertilized eggs in the controls was used to estimate the median effect concentration (EC50) of the reference toxicant (copper sulfate). The EC50 is the concentration where there is a 50 percent reduction in fertilization relative to the controls. The Spearman-Karber method estimates the EC50 with 95 percent confidence limits.

Algal reproduction data (mean number of cystocarps, n = 3) were analyzed on untransformed data using ANOVA and Dunnett's multiple range test (if the ANOVA was significant, $P < 0.05$) to compare responses of organisms (reduction in mean number of cystocarps) in test concentrations with responses in the control water. The validity of the homogeneity of variance assumption was tested by Bartlett's test.

The no observed effect concentration (NOEC) is defined as the highest concentration where a statistically significant response was not observed when compared with the controls. The lowest observed effect concentration (LOEC) is defined as the lowest concentration where a statistically significant response was observed when compared with the controls.

RESULTS

Sample Characterization

The results of sample characterizations are presented in Table 1 for the wet weather sampling event (May 1989) and in Table 2 for the dry weather sampling event (August 1989). When the dissolved oxygen level in a sample was $\leq$ 4.0 mg/L, the sample was aerated prior to use in toxicity tests to raise the DO to an acceptable level ($\geq$5.0 mg/L). The following samples, collected during the May sampling, required aeration: the Field's Point and BVDC STP bypass flow samples, CSO 9, and CSO 10.

Total residual chlorine (TRC) was greater than 0.1 mg/L in the

TABLE 1. RESULTS OF SAMPLE CHARACTERIZATIONS AND PREPARATIONS FOR NARRAGANSETT BAY WET WEATHER SAMPLES RECEIVED ON MAY 11, 1989.

Sample Description	Parameter					Maximum Concentration Tested (% Sample)	
	Temp. (C)	Sal. (o/oo)	DO (mg/l)	pH	TRC (mg/l)	Arbacia	Champia
Sp-4, Pre-Storm	1.0	12	9.7	7.2	0.1	80	50
Sp-4, Bypass	3.4	4	8.8	7.1	0.05	73	50
Sp-4, Peak	5.5	14	8.4	7.4	0.05	74	50
Sp-5, Pre-Storm	1.4	10	8.8	7.4	0.00	79	50
Sp-5, Bypass	8.6	13	9.4	7.2	0.00	81	50
Sp-5, Peak	9.0	11	9.2	7.4	0.01	77	50
Sp-6, Pre-Storm	7.9	12	9.2	7.2	0.00	78	50
Sp-6, Bypass	8.6	14	9.0	7.4	0.00	79	50
Sp-6, Peak	4.1	18	9.0	7.6	0.00	82	50
Sp-8, Pre-Storm	No sample delivered						
Sp-8, Bypass	4.1	13	9.5	7.5	0.00	80	50
Sp-8, Peak	8.3	20	9.8	7.6	0.00	84	50
FPBoil,Pre-Storm	9.3	14	8.7	7.2	0.63	81	50
FPBoil, Bypass	5.8	13	8.2	7.05	0.43	81	50
FPBoil, Peak	5.3	15	8.3	7.2	0.34	82	50
FP STP-A	4.7	0	7.6	6.6	1.09	Combined A+B	
FP STP-B	6.8	0	8.2	6.4	1.25	71	50
FP STP, Bypass-A	7.6	0	1.8*	6.6	0.00	Combined A+B+C	
FP STP, Bypass-B	8.9	0	2.4*	6.6	0.00	Combined A+B+C	
FP STP, Bypass-C	7.4	1	1.6*	6.7	0.00	71	50
BVDC STP-A	4.2	0	5.8	6.9	0.20	Combined A+B	
BVDC STP-B	3.6	0	8.5	6.8	5.08	71	50
BVDC STP,Bypass	5.7	0	4.0*	6.8	0.00	71	50
CSO 9-A	5.0	0	2.0*	6.35	0.00	Combined A+B	
CSO 9-B	9.2	0	3.6*	6.45	0.00	71	50
CSO 10-A	7.4	0	2.2*	6.7	0.00	Combined A+B	
CSO 10-B	5.6	0	2.0*	6.65	0.00	71	50

*Sample aerated before use in toxicity tests.

Temp. = temperature
Sal. = salinity
DO = dissolved oxygen
TRC = total residual chlorine

TABLE 2. RESULTS OF SAMPLE CHARACTERIZATIONS AND PREPARATIONS FOR NARRAGANSETT BAY DRY WEATHER SAMPLES RECEIVED ON 3 AUGUST 1989.

Sample Description	Parameter					Maximum Concentration Tested (% Sample)	
	Temp. (C)	Sal. (o/oo)	DO (mg/l)	pH	TRC (mg/l)	Arbacia	Champia
River Samples							
Spray 4	5.0	25	9.4	7.2	0.00	93	50
Spray 5	8.2	26	7.6	7.3	0.00	93	50
Spray 6	8.5	27	10.2	7.8	0.00	92	50
Spray 7	6.6	25	9.6	8.2	0.00	92	50
Spray 8	6.1	27	9.8	8.2	0.00	95	50
Spray 10	8.0	30	9.0	8.4	0.00	100	50
STP Effluent Samples							
Field's Pt STP	11.4	4	7.6	7.0	0.21	73	50
BVDC STP	10.1	3	7.4	7.4	0.33	72	50

Temp. = temperature
Sal. = salinity
DO = dissolved oxygen
TRC = total residual chlorine

following samples:

Field's Point STP (1.09 - 1.25 mg/L),
Field's Point boil (0.34 - 0.63 mg/L),
BVDC STP (0.20 and 5.08 mg/L).

The dissolved oxygen levels of samples collected during the dry weather sampling were acceptable ($\geq$ 5.0 mg/L) in all samples received. TRC was 0.21 mg/L for the Field's Point STP sample and 0.33 mg/L for the BVDC STP sample. The salinity of samples collected during the wet weather sampling was generally lower than the salinity of samples collected during the dry weather sampling.

Sea Urchin (Arbacia punctulata) Fertilization Tests

Wet Weather Study

Results of the sea urchin fertilization tests completed during the 11 May 1989 wet weather study are presented in Table 3. Six dilution-series tests were completed and fourteen river (ambient water) samples were tested with no dilution beyond the minimum that was necessary for salinity adjustment. Each dilution series included seven concentrations. However, due to apparent test vial contamination or a procedural error, the lowest concentration tested (1 percent sample) resulted in very poor fertilization, whereas fertilization did occur in higher test concentrations. Therefore the data for the 1 percent concentration were considered suspect and omitted from the statistical analysis of each dilution series test. Six concentrations were compared with the controls to identify concentrations where the number of unfertilized eggs was significantly greater ($P < 0.05$) than the controls. For the

TABLE 3. RESULTS OF SEA URCHIN (Arbacia punctulata) FERTILIZATION TEST WITH NARRAGANSETT BAY WET WEATHER SAMPLES RECEIVED ON 11 MAY, 1989

Treatment (% Sample)	Percent Eggs Fertilized in Sample: Fields Point STP	Fields Point STP Bypass	BVDC STP	BVDC STP STP Bypass
SW Control(a)	60	81	60	81
2	83	67	67	52*
4	83	57*	1*	26*
9	29*	15*	1*	2*
18	1*	1*	1*	0*
35	1*	1*	1*	0*
71	0*	1*	0*	1*

Treatment (% Sample)	Percent Eggs Fertilized in Sample: CSO 9	CSO 10
SW Control	80	80
2	80	66
4	80	83
9	64*	65
18	33*	5*
35	2*	3*
71	1*	1*

River Station Sample	Percent Fertilized	River Station Sample	Percent Fertilized
SW Control	60	SW Control	80
Spray 4, Pre-Storm	80	Spray 8, Pre-Storm	Not tested
Spray 4, Bypass	60	Spray 8, Bypass	87
Spray 4, Peak Flow	73	Spray 8, Peak Flow	82
Spray 5, Pre-Storm	55	FP Boil, Pre-Storm	2
Spray 5, Bypass	39	FP Boil, Bypass	0
Spray 5, Peak Flow	69	FP Boil, Peak Flow	1
Spray 6, Pre-Storm	68		
Spray 6, Bypass	71		
Spray 6, Peak Flow	50		

* Indicates that the number of unfertilized eggs were significantly greater than in the SW control.
(a) SW = seawater

controls, the number of fertilized eggs ranged from 60 to 81 percent of the eggs examined. The EC50 for the reference toxicant (copper sulfate) was 5.36 ug/L (95 percent confidence limits 5.08 - 5.65 ug/L).

None of the river station samples were shown to be significantly different from the controls with respect to sea urchin fertilization, with the exception of the Field's Point boil samples collected during the pre-storm, bypass flow, and peak flow periods. In the Field's Point boil samples, at least 98 percent of the eggs were unfertilized, demonstrating high toxicity to sea urchin fertilization. The measured TRC of these samples ranged from 0.34 to 0.63 mg/L.

In each of the five dilution-series tests, significant increases in the number of unfertilized eggs relative to the controls were observed. The NOEC ($P < 0.05$) and the LOEC ($P < 0.05$) for these samples were as follows:

WET WEATHER CHRONIC VALUES, Arbacia punculata

Sample Description	NOEC (Percent Sample)	LOEC (Percent Sample)
Field's Point STP	4	9
Field's Point Bypass	2	4
BVDC STP	2	4
BVDC Bypass	<2	2
CSO 9	4	9
CSO 10	9	18

These samples may be ranked in the following order of increasing toxicity to sea urchin fertilization:

CSO 10 (least toxic)
Field's Point STP / CSO 9
BVDC STP / Field's Point during bypass flow
BVDC STP during bypass flow (most toxic)

It is interesting to note that both the Field Point STP bypass and the BVDC STP bypass effluent samples were more toxic than STP flow prior to bypass activation. It is also interesting to note that although the bypass samples were more toxic, TRC was not detected in these samples, whereas TRC was a component of the STP post-chlorinated samples.

Dry Weather Study

Results of sea urchin fertilization tests which were completed during the dry weather period (August 1989) are presented in Table 4. Dilution-series tests were completed, on Field's Point and BVDC STP samples. Six river station samples were tested with no dilution beyond the minimum necessary for salinity adjustment. Each dilution series test included six concentrations of sample. The number of unfertilized eggs in each concentration was compared with the controls. In the controls, 86 to 90 percent of the eggs examined were fertilized. The EC50 of the reference toxicant was 48.4 ug/L (95 percent confidence limits, 45.3 - 51.7 ug/L).

Sea urchin egg fertilization in the river station samples was not shown to be significantly different from the control. A toxic response was identified, however, in the Field's Point STP and BVDC STP samples. The NOEC and LOEC values for these samples were

TABLE 4. RESULTS OF SEA URCHIN (Arbacia punctulata) FERTILIZATION TEST WITH NARRAGANSETT BAY DRY WEATHER SAMPLES RECEIVED ON 3 AUGUST 1989

	Percent Eggs Fertilized in Sample		
Treatment (% Sample)	Fields Point STP	Treatment (% Sample)	BVDC STP
SW Control(a)	86	88	60
2	88	85	67
5	78	88	1*
9	47*	79*	1*
18	9*	62*	1*
36	1*	1*	1*
73	0*	1*	0*

River Station Sample	Percent Fertilized
SW Control	90
Spray 4	90
Spray 5	82
Spray 6	92
Spray 7	86
Spray 8	90
Spray 10	90

* Indicates that the number of unfertilized eggs were significantly greater than in the SW control.
(a) SW = seawater

as follows:

DRY WEATHER CHRONIC VALUES (Arbacia punctulata)

Sample Description	NOEC (Percent Sample)	LOEC (Percent Sample)
Field's Point STP	5	9
BVDC STP	4	9

The Field's Point and the BVDC STP effluent samples collected during the dry weather study were very similar with regard to chronic toxicity to Arbacia punctulata. TRC was measured as 0.21 mg/L and 0.33 mg/L respectively, for the Field's Point and BVDC STP samples.

Marine Alga (Champia parvula) Sexual Reproduction Test

Wet Weather Study

Results of the Champia parvula sexual reproduction tests which were completed during the wet weather sampling event are summarized in Table 5. Six samples were tested as dilution-series tests and fourteen individual river station samples (50 percent dilution only) were tested. Each dilution series test included five sample concentrations. Insufficient numbers of cystocarps developed to evaluate the wet weather samples (Control plants should average 10 or more cystocarps). Therefore, a statistical analysis was not performed on these tests. There appeared to be a trend toward reduction in the numbers of cystocarps relative to the controls in all samples tested. NOEC and LOEC values for Champia parvula were not computed for the wet weather samples.

TABLE 5. RESULTS OF *Champia parvula* SEXUAL REPRODUCTION TESTS WITH NARRAGANSETT BAY WET WEATHER SAMPLES RECEIVED ON 11 MAY 1989

Treatment (% Sample)	$\bar{X}$ Number of Cystocarps in Sample: Fields Point STP	Fields Point STP Bypass	BVDC STP	BVDC STP STP Bypass
SW Control(a)	5.5	2.7	1.8	6.1
0.6	1.1	1.5	0.6	0.6
1.8	3.1	0.9	2.6	0.5
5.4	0.3	0.1	4.2	0.0
16.5	1.1	0.4	0.2	0.3
50.0	0.9	0.9	0.0	0.0

Treatment (% Sample)	$\bar{X}$ Number of Cystocarps in Sample: CSO 9	CSO 10
SW Control	1.8	3.6
0.6	1.2	1.4
1.8	0.3	0.2
5.4	0.1	0.3
16.5	0.0	0.0
50.0	0.1	0.1

River Station Sample	$\bar{X}$ No. Cystocarps	River Station Sample	$\bar{X}$ No. Cystocarps
SW Control	5.0	SW Control	4.3
Spray 4, Pre-Storm	1.9	Spray 8, Pre-Storm	Not tested
Spray 4, Bypass	2.0	Spray 8, Bypass	0.8
Spray 4, Peak Flow	1.9	Spray 8, Peak Flow	1.4
SW Control	5.0	SW Control	5.3
Spray 5, Pre-Storm	1.5	FP Boil, Pre-Storm	2.0
Spray 5, Bypass	5.1	FP Boil, Bypass	5.6
Spray 5, Peak Flow	3.2	FP Boil, Peak Flow	3.1
SW Control	5.3		
Spray 6, Pre-Storm	3.5		
Spray 6, Bypass	1.1		
Spray 6, Peak Flow	1.2		

(a) SW = seawater

Dry Weather Study

During the dry weather sampling event of August 1989, Champia parvula sexual reproduction tests were successfully completed on on a Field's Point STP post-chlorinated effluent sample and a BVDC STP post-chlorinated effluent sample. The results of the Champia parvula dry weather tests are presented in Table 6. The STP samples were tested as a full dilution series with five sample concentrations. Also, six river station samples were tested as a 50 percent dilution of each sample. Appreciation is extended to EPA-ERLN staff and especially to Dr. Glen Thursby (SAIC) and to Mr. Mark Tagliabue (SAIC) for their assistance in preparing for the dry weather Champia tests.

Each treatment level of the dilution series tests and each river sample was compared with the controls to determine whether a significant reduction in the number of cystocarps had occurred. The average number of cystocarps in the controls ranged from 24 to 26 cystocarps per female branch.

Champia parvula was very sensitive to water samples collected during the dry weather sampling period in Narragansett Bay. Each of the six river stations demonstrated a significant reduction in the number of cystocarps when compared with the controls. The Champia plants in the river sample collected off Field's Point showed the greatest reduction in numbers of cystocarps relative to the control. The river station samples may be ranked according to increasing toxicity to Champia parvula reproduction as follows:

TABLE 6. RESULTS OF *Champia parvula* SEXUAL REPRODUCTION TESTS WITH NARRAGANSETT BAY DRY WEATHER SAMPLES RECEIVED ON 3 AUGUST 1989

Treatment (% Sample)	$\bar{X}$ Number of Cystocarps in Sample: Fields Point STP	BVDC STP
SW Control(a)	24.0	27.0
0.6	18.5	18.3*
1.8	21.7	17.3*
5.4	9.7*	17.3*
16.5	0.1*	0.5*
50.0	0 *	0 *

River Station Sample	$\bar{X}$ No. Cystocarps
SW Control	26.0
Spray 4	14.5*
Spray 5	13.5*
Spray 6	7.4*
Spray 7	12.0*
Spray 8	21.5*
Spray 10	21.5*

(a) SW = seawater

Spray 8 (off Gaspie Point) / Spray 10 (off Conimicut Point) (least toxic)
Spray 4 (mouth of Seekonk River, off India Point)
Spray 5 (off Sassafras Point)
Spray 7 (off Sabin's Point)
Spray 6 (off Field's Point) - most toxic.

Toxicity was also identified in the dilution-series tests conducted on the Field's Point STP and the BVDC STP samples. The chronic values identified for the dry weather study were as follows:

DRY WEATHER CHRONIC VALUES (Champia parvula)

Sample Description	NOEC (Percent Sample)	LOEC (Percent Sample)
Field's Point STP	1.8	5.4
BVDC STP	<0.6	0.6

The BVDC STP effluent sample was toxic at the lowest dilution level tested.

DISCUSSION AND CONCLUSIONS

The sea urchin (Arbacia punctulata) fertilization test was used to measure the chronic toxicity of ambient waters and chlorinated STP effluents during wet weather (May 1989) and dry weather (August 1989) seasons. Chronic toxicity of STP bypass flows and CSO discharges to this species was measured during the wet weather period only, when these outputs are active. The sea urchin chronic test procedure was found to provide a reliable and sensitive indication of water quality in representative water samples, especially point-source discharges, collected in Narragansett Bay during both the wet and the dry sampling periods. Although the ambient river samples did not result in a significant

chronic response during either the wet or dry periods, the chronic toxicity of two chlorinated STP effluents can be compared for the two seasonal events.

The Field's Point STP and the BVDC STP chlorinated effluents showed little change in chronic toxicity during the two sampling periods. The chronic values (as percent sample) for Arbacia punctulata for these periods may be compared as follows:

Sample Description	May 1989 Wet Weather NOEC	LOEC	August 1989 Dry Weather NOEC	LOEC
Field's Point STP	4	9	5	9
BVDC STP	2	4	4	9

The BVDC STP sample showed slight improvement during the dry period (NOEC, 2 times higher). This improvement may be due, in part to the fact that one of the two wet weather BVDC STP samples composited had a very high measured TRC (5.08 mg/L). The equal-volume compositing of this sample reduced the TRC to 2.64 mg/L (nominal). Assuming that the TRC is diluted in a linear manner when a dilution series is prepared with seawater, the dilution series for this test can be expressed in terms of TRC concentration (nominal) with the following dilution series: 1.87 (The highest test concentration was 71 percent of the original sample.), 0.94, 0.47, 0.23, 0.12, and 0.06 mg TRC/L. The NOEC and LOEC values for Arbacia punctulata expressed as TRC concentration would then be 0.06 mg/L and 0.12 mg/L, respectively.

While the Arbacia punctulata fertilization test did not show a significant chronic response to ambient river water samples, Champia parvula demonstrated significant sensitivity to all river

samples tested during the August dry weather period. Champia parvula was also very sensitive to the chlorinated STP discharges. We can conclude that the natural condition of the upper Narragansett Bay waters, during the August 1989 sampling event, was not suitable for Champia parvula sexual reproduction.

A sensitivity factor may be derived by comparing the chronic values for Champia parvula with the chronic values for Arbacia punctulata. The greater chronic value was divided by the lesser chronic value, for the two species, to obtain a sensitivity factor. The results were as follows:

Sample Description	SENSITIVITY FACTOR	
	NOEC	LOEC
Field's Point STP	Champia, 2.8 X	Champia, 1.7 X
BVDC STP	Champia, >6.7 X	Champia, 15.0 X

The Champia parvula chronic response was 1.7 to 15 times more sensitive than the Arbacia punctulata chronic response. The sea urchin fertilization test was sensitive to point-source pollution inputs, while the algal reproduction test was sensitive not only to point-source inputs, but also to the prevailing ambient water quality of the upper reaches of Narragansett Bay during summer.

REFERENCES

Weber, C.I. et al. (eds). 1988. Short-term methods for estimating the chronic toxicity of effluents and receiving waters to marine and estuarine organisms. EPA-600/4-87/028. Environmental Monitoring and Support Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. 417 pp.

APPENDIX A

CORRESPONDENCE BETWEEN CES SAMPLE DESCRIPTIONS AND SAMPLES RECEIVED AND TESTED

APPENDIX A

CORRESPONDENCE BETWEEN CES SAMPLE DESCRIPTIONS AND SAMPLES RECEIVED AND TESTED

WET WEATHER SAMPLES, MAY 1989

RIVER STATIONS

Pre-Storm	During Bypass Activation	During Peak Flow
#4 Spray 4 Run 1	#17 Spray 4 Top Run 4	#8 Spray 4 Top Run 6
#5 Spray 5 Run 1	#10 SP 5 Top Run 4	#9 SP 5 Top Run 6
#6 Spray 6 Run 1	#28 Spray 6 Top Run 4	#23 Spray 6 Top Run 6
Not collected	#27 Spray 8 Top Run 4	#7 Spray 8 Top Run 6
#3 Boil FP Run A	#16 FP Boil Run C	#14 NBC Boil Run F

STP STATIONS

Fields Point: Combined sample #12 (NBC post chlor) with sample #25 (FP post chlor).

BVDC: Combined sample #20 (BVDC Effl Run C) with sample #22 (BVDC Run 6).

STP STATIONS DURING BYPASS ACTIVATION

Fields Point: Combined sample #13 (NBC FP Bypass Run D) with sample #15 (FP Bypass time 1:35) and with sample #26 (Fields Point Bypass Run B).

BVDC: Sample #21 (BVDC Bypass time 1500).

CSO STATIONS

CSO 9: Combined sample #19 (CSO 9 Run B time 1412) with sample #24 (CSO 9 Run C time 1633).

CSO 010: Combined sample #11 (CSO D Sta S-010) with sample #18 (CSO D Sta S-010 time 0135)

APPENDIX A

CORRESPONDENCE BETWEEN CES SAMPLE DESCRIPTIONS AND SAMPLES RECEIVED AND TESTED

DRY WEATHER SAMPLES, AUGUST 1989

RIVER STATIONS

Spray 4 - #4 at mouth of Seekonk River off India Point.
Spray 5 - #5 mid-channel off gas pipeline. STP effluent off Sassafras Point.
Spray 6 - #6 mid-channel off Field's Point.
Spray 7 - #7 off Sabin's Point between buoys, mid-channel.
Spray 8 - #8 off Gaspie Point, mid-channel.
Spray 10 - #10 off Conimicut Point Light, mid-channel.

STP STATIONS

Fields Point: Combined six samples marked at the following times: 1100, 3:55, 6:30 (four samples).

BVDC: Combined four samples marked at the following times: 9:35, 1255 (two samples), 4:35.