$\label{eq:Attachment A}$  Charles River Temperature Limits by Time of Year in the Zone of Passage and Habitat (ZPH)^1

Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
March 30 - April 1*	Not to exceed 4 hour average <sup>3</sup> of 13.3°C (56.0°F) at each Monitoring Point <sup>4</sup> in the ZPH.	Not less than 5.0 mg/l <sup>5.6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations <sup>7</sup> 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
April 2 - April 14*	Not to exceed a 4 hour average of 16.1°C (61.0°F) at each Monitoring Point.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
April 15 - April 30**	Not to exceed 4 hour average of 18.3°C (65.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
May 1 - May 10**	Not to exceed 4 hour average of 19.1°C (66.4.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
May 1 - May 14**	Not to exceed a 4 hour average of 18.3°C (65.0°F) at each Monitoring Point.	Not less than 5.0 mg/l <sup>5.6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Station 8. Each Monitoring Points located at 2.0 ft. and 6.0 ft. must meet this temperature limit.
May 11 - May 14**	Not to exceed a 4 hour average of 20.0°C (68.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5.6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, and 7. Each Monitoring Point in the ZPH must meet this temperature limit.

Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
May 15 - May 22**	Not to exceed a 4 hour average of 20.0°C (68.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
May 23 - May 31**	Not to exceed a 4 hour average of 21.1°C (70.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.
June 1 to June 7**	Not to exceed a 4 hour average of 21.1°C (70.0°F) at each Monitoring Point.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Station 8. Each Monitoring Point, located at 2.0 ft. and 6.0 ft., must meet this temperature limit.
June 1 - June 7**	Not to exceed a 4 hour average of 22.2°C (72.0°F) at each Monitoring Point in the ZPH	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, and 7. Each Monitoring Point in the ZPH must meet this temperature limit.
June 8 - June 11	Not to exceed a 4 hour average of 23.9°C (75.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8. Each Monitoring Point in the ZPH must meet this temperature limit.

Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
June 12 - Oct. 31	Not to exceed a 4 hour average of 28°C (83.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, 6, 7 and 8.
Additional Special Day-Time Limits:	A compliance temperature of 81° F or less shall be met in each of the four daytime temperature-averaging periods (4 am – 8 am; 8 am – 12 noon; 12 noon – 4 pm; and 4 pm – 8 pm) at either the six- or the twelve-foot monitoring depths at Monitoring Stations 2, 3, and 7.	Not less than 5.0 mg/l <sup>5.6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3 and 7
Additional Special Night-Time Limits:	A compliance temperature of 81° F or less shall be met for at least one of the two nighttime temperature-averaging periods (8 pm – 12 midnight; or 12 midnight – 4 am) at the two-foot monitoring depth at Monitoring Stations 2, 3,& 7. These nighttime conditions do not have to be met within the same four-hour block at all three monitoring stations.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, and 7
November 1 to March 29 (chill period")* * *	Not to exceed a 4 hour average of 10.0°C (50.0°F) at each Monitoring Point in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, 4, 5, and 6

Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
The Chill Period Shall Begin on:  1) Nov. 1, providing that 1) Max. Temperature provisions are met (see next column)  2) if not begun under condition 1) above, then between November 1 and November 14, when 2) Max Temperature provisions are met (see next column)	(The numbered items below correspond to the same numbered items in the "Time of Year" column)  1) Maintain water temperatures in the In-Zone transect ZPH, based on a temperature average of the 2.0 ft. and 6.0 ft .Monitoring Points over a four hour period, at or below 10.0°C (50.0°F) for three consecutive days.  2) Condition 1 (see Time of Year column) has been met, or when Monitoring Station 1 temperature averaged from the 2.0 ft. and 6.0 ft. Monitoring Points over a four hour period, is at or below 10.0°C (50.0°F) for three consecutive days.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH.	Monitoring Stations 2, 3, (4, 5 and 6, if in the ZPH) based on a temperature average of Monitoring Points at 2.0 ft. and 6.0 ft.
3) if not begun under conditions 1) or 2) above, then beginning on November 15, provided that 3) Max Temp. provisions are met (see next column)	3) if conditions 1) and 2) have not been met, maintain the river temperatures in the ZPH at no more than 10.0°C (50.0°F). If background temperature exceeds 10.0°C (50.0°F), ZPH may rise to 1.1°C (2.0°F) above background temperature. Background temperature, <b>for this purpose only</b> , is defined as the warmer of the 2.0 ft. or 6.0 ft. Monitoring Points located at Monitoring Station 1. Once Max Temperature conditions 1) or 2) are met, this condition no longer applies.		

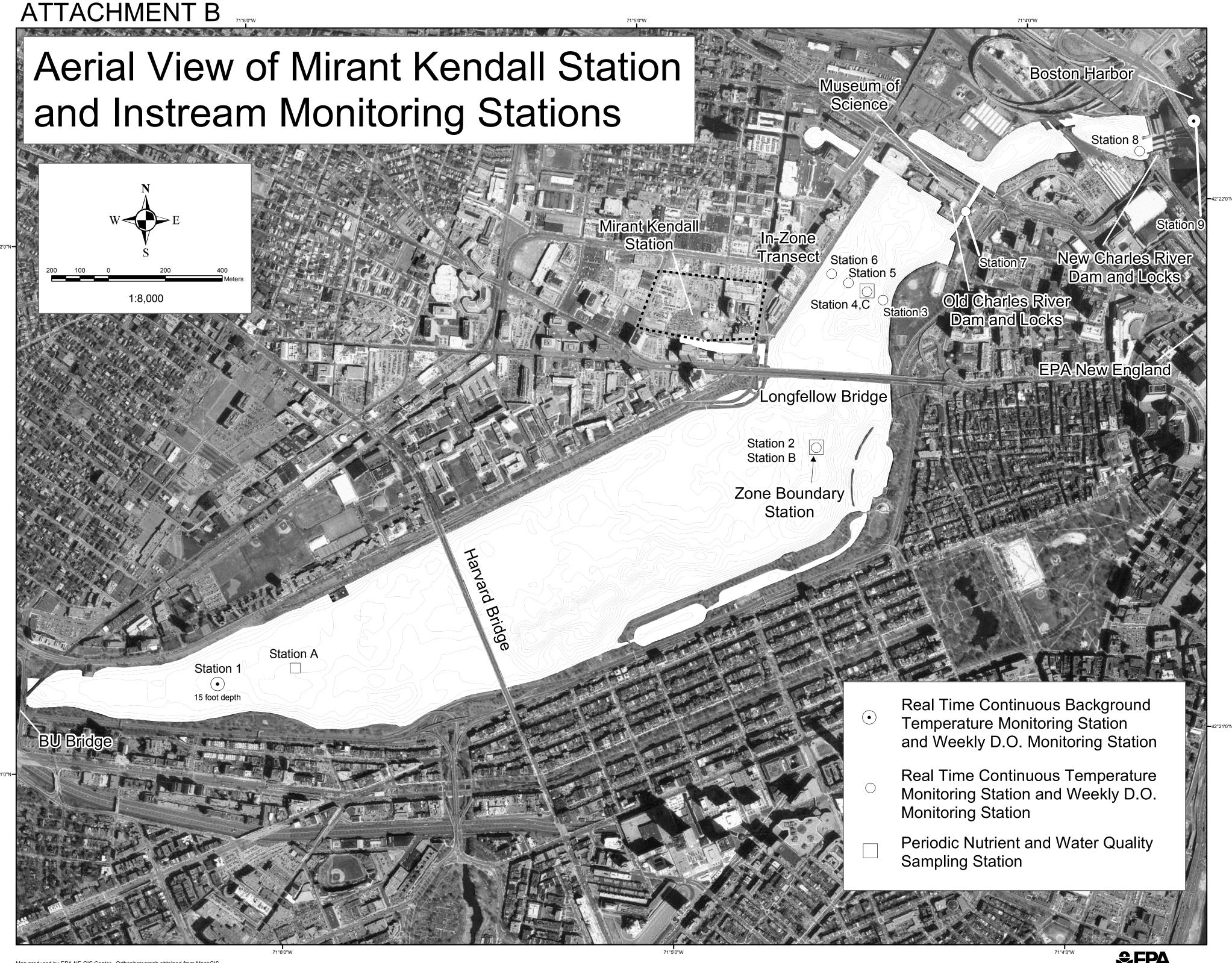
Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
The Chill Period will end on March 29 <sup>th</sup> , when 149 days have met the Max. Temperature provisions:  1) if by March 29 <sup>th</sup> the temperatures have not met Max	1) maintain the river temperatures in the ZPH at no more than 10.0°C (50.0°F). If background temperature exceeds 10.0°C (50.0°F), ZPH may	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in	Monitoring Stations 2, 3, (4, 5 and 6, if in the ZPH) based on a temperature average of Monitoring Points at 2.0 ft.
Temperature provisions for 149 days, then the Facility will operate in accordance with 1) Max. Temperature provisions (see next column) until 149	rise to 1.1°C (2.0°F) above background temperature. Background temperature, <b>for this purpose only</b> , is defined as the warmer of the 2.0 ft. or 6.0 ft. Monitoring Points located at Monitoring Station 1.	the ZPH.	and 6.0 ft.
days are met  2) The chill period will end after being in effect for 149 days or on April 13 <sup>th</sup> , whichever comes first			

Time of Year	Max. Temperature <sup>2</sup>	Dissolved Oxygen	Location
All Year	1) The maximum temperature differential limit of 2.8°C (5°F) or "Delta T of 2.8 °C (5°F)" will be determined by comparing a) the 24 hour block time average (00:00 [midnight]-23:59) of temperatures recorded at Monitoring Points at 2.0 ft and 6.0 ft. (averaged together) at Station 1 with b) the 24 hour block average (00:00 [midnight]-23:59) of the temperatures recorded at Monitoring Points at 2.0 ft. and 6.0 ft. (averaged together) at each of the Monitoring Stations in the ZPH.	Not less than 5.0 mg/l <sup>5,6</sup> as the most recent instantaneous value recorded at least weekly at each Monitoring Point in the ZPH	Compare the average of the 2.0 ft. and 6.0 ft. Monitoring Points at Monitoring Station 1, with the the 2.0 ft and 6.0 ft average at each of Monitoring Stations 2. 3, 4 and 8 in the ZPH. At Station 7 only, the average of the 6.0 ft and 12.0 ft Monitoring Points (instead of the 2.0 ft and 6.0 ft average) may be compared with the Monitoring Station 1 average, if this will result in compliance with the Delta T limit.

- \* The Start Date of this temperature limit will be the day after the yellow perch chill period ends, if later than March 29.
- \*\* If the Monitoring Station 1 (background station) temperature, averaged from the 2.0 ft and 6.0 ft Monitoring Points over a four hour period, exceeds the established limits identified for a given time period, then the Station 1 average temperature becomes the applicable temperature limit. Exception: For six non-consecutive 24 hour periods only during the time-frame from April 15 to June 7, if the Monitoring Station 1 average temperature is at or above the prescribed limit, the Facility will be allowed to operate such that the river temperatures downstream of Monitoring Station 1 in the ZPH are 1.1°C (2.0°F) above background up to a maximum temperature of 22.2°C (72.0°F); except for the June 1 to June 7 time period, where the temperature may be 1.1°C (2.0°F) above the Monitoring Station 1 average temperature up to a maximum temperature of 23.3°C (74.0°F). To ensure that all six (6) 24 hour periods are not used within a brief time frame, only three (3) exceedances will be allowed in any four week period. Unused days will not be carried over to future years.
- \*\*\* If "Chill Period" temperature has not been met by November 1, then Chill Period Limits will be in effect when the Monitoring Station 1 (background station) temperature averaged from the 2.0 ft. and 6.0 ft. Monitoring Points over a four hour period, reach 10.0°C (50.0°F) or below for three consecutive days.

### Footnotes:

- 1. The "Zone of Passage and Habitat" (ZPH) is the area of the lower Charles River Basin that is viable habitat (temperature and dissolved oxygen levels causing no acute or chronic effects), spanning from the upstream location of the Zone Boundary Transect to the downstream location of the New Charles River Dam and Locks (Attachment B). The ZPH is defined by compliance with temperature and dissolved oxygen limits at the following Monitoring Points: All Monitoring Points at Station 2 must be met at all times. This station is located upstream of the Longfellow Bridge, approximately half the distance between the Boston and Cambridge shores of the Charles River. The ZPH also includes, at all times, all Monitoring Points at Station 3 (Boston side). The ZPH includes, at all times, a sufficient number of Monitoring Points at Stations 5 and 6 such that at least ½ of the total number of Monitoring Points that meet the dissolved oxygen limit of 5.0 mg/l in the In-Zone Transect (Stations 3, 4, 5 and 6) meet the temperature limit in effect. These Monitoring Points must be contiguous with Monitoring Points at Station 4. In addition, the ZPH includes, at all times that Monitoring Station 7 (located at the Science Museum lock) is required, at least two contiguous Monitoring Points (2.0 ft. and 6.0 ft., or 6.0 ft and 12.0 ft.). The ZPH also includes, at all times that Monitoring Station 8 (upstream of the New Charles River Dam and Locks) is required, the 2.0 ft. and 6.0 ft. Monitoring Point.
- 2. Should the 4 hour average temperature of the average of the 2.0 ft. and 6.0 ft. Monitoring Points of Monitoring Station 1 near the Boston University Bridge (Background Station) exceed any of the applicable temperature limits specified in this table for the corresponding time period, the temperature at Monitoring Station 1 will become the maximum temperature limit until the average temperature at 2.0 ft. and 6.0 ft. of the Monitoring Points at Monitoring Station 1 fall below the applicable maximum temperatures herein.
- 3. The 4 hour average consists of a minimum of one instantaneous measurement, at evenly spaced time intervals and taken within each of the four discrete one hour time periods (minimum of four total readings). If more than one reading is used within a discrete hour, the readings must be taken at evenly spaced time intervals within the hour (e.g. every 15 minutes) and the same number of readings, at the same temporal spacing, must be used for all hours within the four hour average block for the calculation of the four hour average temperature. Four hour averages shall be reported daily for the following four hour time blocks: day-time blocks 4:00 7:59, 8:00 11:59, 12:00 15:59, 16:00 19:59 and night-time blocks 20:00 23:59, 0:00 3:59.
- 4. A Monitoring Point is an underwater probe at a discrete depth which records temperature and other water quality parameters when specified.
- 5. Dissolved oxygen concentrations are for all depths except where indicated.
- 6. A dissolved oxygen concentration below 5 mg/l, when measured at a Monitoring Point required to be in the ZPH, is not a violation of the permit limit. If the dissolved oxygen concentration is less than 5 mg/l at any Monitoring Point, then that Monitoring Point is not considered to be located in suitable habitat. Therefore, that Monitoring Point will no longer be a temperature compliance point. In the case of the In-Zone Transect (Monitoring Stations 3, 4,5 and 6) a Monitoring Point with a dissolved oxygen level below 5 mg/l will not be used in the calculation to verify that at least ½ of the Monitoring Points of the In-Zone Transect meet the temperature limit in effect for a corresponding time period.
- 7. A Monitoring Station is a vertical array of temperature and other sensors suspended by a buoy or attached to a permanent structure in the Charles River.



### ATTACHMENT C1

# FRESHWATER CHRONIC TOXICITY TEST PROCEDURE AND PROTOCOL

# I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable chronic (and modified acute) toxicity tests on three samples collected during the test period. The following tests shall be performed in accordance with the appropriate test protocols described below:

- Daphnid (Ceriodaphnia dubia) Survival and Reproduction Test.
- Fathead Minnow (Pimephales promelas) Larval Growth and Survival Test.

Chronic toxicity data shall be reported as outlined in Section VIII. The chronic fathead minnow and daphnid tests can be used to calculate an LC50 at the end of 48 hours of exposure when both an acute (LC50) and a chronic (C-NOEC) test is specified in the permit.

# II. METHODS

Methods to follow are those recommended by EPA in:

Lewis, P.A. et al. Short Term Methods For Estimating The Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms, Third Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, Cincinnati, OH. July 1994, EPA/600/4-91/002.

Any exceptions are stated herein.

### III. SAMPLE COLLECTION

For each sampling event, three discharge samples shall be collected. Fresh samples are necessary for Days 1, 3, and 5 (see Section V. for holding times). The initial sample is used to start the test on Day 1, and for test solution renewal on Day 2. The second sample is collected for use at the start of Day 3, and for renewal on Day 4. The third sample is used for renewal on Days 5, 6, and 7 (or until termination for the Ceriodaphnia dubia test). The initial (Day 1) sample will be analyzed chemically (see Section VI). Day 3 and 5 samples will be held until test completion. If either the Day 3 or 5 renewal sample is of sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions for either species, then a chemical analysis shall be performed on the appropriate sample(s) as well.

Aliquots shall be split from the samples, containerized and preserved (as per 40 CFR Part 136) for chemical and physical analyses. The remaining samples shall be measured for total residual chlorine and dechlorinated (if detected) in the laboratory using sodium thiosulfate for subsequent

toxicity testing. (<u>Note that EPA approved test methods require that samples collected for metals analyses be preserved immediately after collection.</u>) Grab samples must be used for pH, temperature, and total residual chlorine (as per 40 CFR Part 122.21).

Standard Methods for the Examination of Water and Wastewater also describes dechlorination of samples (APHA, 1992). Dechlorination can be achieved using a ratio of 6.7 mg/L anhydrous sodium thiosulfate to reduce 1 mg/L chlorine. A thiosulfate control (maximum amount of thiosulfate in lab control or receiving water) should also be run.

All samples held overnight shall be refrigerated at 4°C.

# IV. DILUTION WATER

Grab samples of dilution water used for chronic toxicity testing shall be collected from the receiving water at a point upstream of the discharge free from toxicity or other sources of contamination. Avoid collecting near areas of obvious road or agricultural runoff, storm sewers or other point source discharges. An additional control (0% effluent) of a standard laboratory water of known quality shall also be tested.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate standard dilution water of known quality with a hardness, pH, conductivity, alkalinity, organic carbon, and total suspended solids similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. Written requests for use of an alternate dilution water should be mailed with supporting documentation to the following address:

Director
Office of Ecosystem Protection
U.S. Environmental Protection Agency, Region 1
One Congress Street
Suite 1100 (CAA)
Boston, MA 02114-2023

It may prove beneficial to have the dilution water source screened for suitability prior to toxicity testing. EPA strongly urges that screening be done prior to set up of a full definitive toxicity test any time there is question about the dilution water's ability to support acceptable performance as outlined in the 'test acceptability' section of the protocol. See Section 7 of EPA/600/4-89/001 for further information.

# V. TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA

EPA New England requires that fathead minnow tests be performed using <u>four</u> (not three) replicates of each control and effluent concentration because the non-parametric statistical tests cannot be used with data from only three replicates. Also, if a reference toxicant test was being performed concurrently with an effluent or receiving water test and fails, both tests must be repeated.

The following tables summarize the accepted daphnid and fathead minnow toxicity test conditions and test acceptability criteria:

# EPA NEW ENGLAND RECOMMENDED EFFLUENT TOXICITY TEST CONDITIONS FOR THE DAPHNID, <u>CERIODAPHNIA DUBIA</u>, SURVIVAL AND REPRODUCTION TEST $^1$

1. Test type:	Static, renewal
2. Temperature (°C):	$25 \pm 1^{\circ}$ C
3. Light quality:	Ambient laboratory illumination
4. Photoperiod:	16 hr. light, 8 hr. dark
5. Test chamber size:	30 mL
6. Test solution volume:	15 mL
7. Renewal of test solutions:	Daily using most recently collected sample
8. Age of test organisms:	Less than 24 hr.; and all released within an 8 hr. period of each other.
9. Number of neonates per test chamber:	1
10. Number of replicate test chambers per treatment:	10
11. Number of neonates per test concentration:	10
12. Feeding regime:	Feed 0.1 ml each of YCT and concentrated algal suspension per exposure chamber daily.
13. Aeration:	None
14. Dilution water: <sup>2</sup>	Receiving water, other surface water, synthetic soft water adjusted to the hardness and alkalinity of the receiving water (prepared using either Millipore Milli-Q <sup>R</sup> or equivalent deionized water and reagent

grade chemicals according to EPA chronic toxicity test manual) or deionized water combined with mineral water to appropriate hardness.

15. Effluent concentrations:<sup>3</sup>

5 effluent concentrations and a control. An additional dilution at the permitted effluent concentration (% effluent) is required if it is not included in the dilution series.

16. Dilution factor:

 $\geq 0.5$ 

17. Test duration:

Until 60% of control females have three broods (generally 7 days and a maximum of

8 days).

18. End points:

Survival and reproduction

19. Test acceptability:

80% or greater survival and an average of 15 or more young/surviving female in the control solutions. At least 60% of surviving females in controls must produce three

broods.

20. Sampling requirements:

For on-site tests, samples are collected daily and used within 24 hr. of the time they are removed from the sampling device. For off-site tests a minimum of three samples are collected (i.e. days 1, 3, 5) and used for renewal (see Sec. III). Off-site tests samples must be first used within 36 hours of

collection.

21. Sample volume required:

Minimum 1 liter/day

# Footnotes:

- Adapted from EPA/600/4-91/002.
- Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.
- When receiving water is used for dilution, an additional control made up of standard laboratory dilution water (0% effluent) is required.

# EPA NEW ENGLAND RECOMMENDED EFFLUENT TEST CONDITIONS FOR THE FATHEAD MINNOW (PIMEPHALES PROMELAS) LARVAL SURVIVAL AND GROWTH TEST $^{\rm I}$

1. Test type: Static, renewal 2. Temperature (°C):  $25 + 1^{\circ}C$ 3. Light quality: Ambient laboratory illumination 4. Photoperiod: 16 hr. light, 8 hr. dark 5. Test chamber size: 500 mL minimum 6. Test solution volume: Minimum 250 mL/replicate 7. Renewal of test concentrations: Daily using most recently collected sample. Newly hatched larvae less 8. Age of test organisms: than 24 hr. old 9. No. larvae/test chamber 15 (minimum of 10) and control: 10. No. of replicate chambers/ 4 concentration: 11. No. of larvae/concentration: 60 (minimum of 40) 12. Feeding regime: Feed 0.1 g newly hatched, distilled waterrinsed Artemia nauplii at least 3 times daily at 4 hr. intervals or, as a minimum, 0.15 g twice daily, 6 hrs. between feedings (at the beginning of the work day prior to renewal, and at the end of the work day following renewal). Sufficient larvae are added to provide an excess. Larvae fish are not fed during the final 12 hr. of the test. Siphon daily, immediately before test 13. Cleaning: solution renewal.

14. Aeration: None, unless dissolved oxygen (D.O.)

concentration falls below 4.0 mg/L. Rate

should be less than 100 bubbles/min.

15. Dilution water: Receiving water, other surface water,

synthetic soft water adjusted to the hardness and alkalinity of the receiving water (prepared using either Millipore Milli-Q<sup>R</sup> or equivalent deionized and reagent grade chemicals according to EPA chronic toxicity

test manual) or deionized water combined with mineral water to appropriate hardness.

16. Effluent concentrations:<sup>3</sup> 5 and a control. An additional dilution at

the permitted effluent concentration (% effluent) is required if it is not included in

the dilution series.

17. Dilution factor:  $\geq 0.5$ 

18. Test duration: 7 days

19. End points: Survival and growth (weight)

20. Test acceptability: 80% or greater survival in controls: average

dry weight per control larvae equals or

exceeds 0.25 mg.

21. Sampling requirements: For on-site tests samples are collected and

used within 24 hours of the time they are removed from the sampling device. For off-site tests a minimum of three samples are collected (i.e. days 1, 3, 5) and used for renewal (see Sec.IV). Off-site tests samples

must be first used within 36 hours of

collection.

22. Sample volume required: Minimum 2.5 liters/day

# Footnotes:

Adapted from EPA/600/4-91/002.

- Standard dilution water must have hardness requirements to generally reflect characteristics of the receiving water.
- When receiving water is used for dilution, an additional control made up of standard laboratory or culture water (0% effluent) is required.

# VI. CHEMICAL ANALYSIS

As part of each daily renewal procedure, pH, specific conductance, dissolved oxygen, and temperature must be measured at the beginning and end of each 24-hour period in each dilution and the controls. It is also recommended that total alkalinity and total hardness be measured in the control and highest effluent concentration on the Day 1, 3, and 5 samples. The following chemical analyses shall be performed for each sampling event.

			Minimum Quantificatio
			n Level
<u>Parameter</u>	<b>Effluent</b>	<u>Diluent</u>	(mg/L)
Hardness *1	X	X	0.5
Alkalinity	X	X	2.0
рН	X	X	
Specific Conductance	X	X	
Total Solids and Suspended Solids	S X	X	
Ammonia	X	X	0.1
Total Organic Carbon	X	X	0.5
Total Residual Chlorine (TRC) *2	X	X	0.05
Dissolved Oxygen	X	X	1.0
Total Metals			
Cd	X	X	0.001
Cr	X	X	0.005
Pb	X	X	0.005
Cu	X	X	0.0025
Zn	X	X	0.0025
Ni	X	X	0.004
Al	X	X	0.02
Mg, Ca	X	X	0.05

# Superscripts:

or use USEPA Manual of Methods Analysis of Water and Wastes, Method 330.5.

Method 2340 B (hardness by calculation) from APHA (1992) <u>Standard Methods for the</u> Examination of Water and Wastewater. 18th Edition.

Either of the following methods from the 18th Edition of the APHA <u>Standard Methods</u> for the Examination of Water and Wastewater must be used for Total Residual Chlorine analyses:

<sup>-</sup>Method 4500-CL E Low Level Amperometric Titration (the preferred method);

<sup>-</sup>Method 4500-CL G DPD Colorimetric Method.

# VII. TOXICITY TEST DATA ANALYSIS

# LC50 Median Lethal Concentration (Determined at 48 Hours)

Methods of Estimation:

- Probit Method
- Spearman-Karber
- Trimmed Spearman-Karber
- Graphical

Reference the flow chart on page 84 or page 172 of EPA 600/4-91/002 for the appropriate method to use on a given data set.

# Chronic No Observed Effects Concentration (C-NOEC)

Methods of Estimation:

- Dunnett's Procedure
- Bonferroni's T-Test
- Steel's Many-One Rank Test
- Wilcoxin Rank Sum Test

Reference the flow charts on pages 50, 83, 96, 172, and 176 of EPA 600/4-91/002 for the appropriate method to use on a given data set.

In the case of two tested concentrations causing adverse effects but an intermediate concentration not causing a statistically significant effect, report the C-NOEC as the lowest concentration where there is no observable effect. The definition of NOEC in the EPA Technical Support Document only applies to linear dose-response data.

# VIII. TOXICITY TEST REPORTING

A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis on chain-of-custody; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended. Reference toxicant test data should be included.
- All chemical/physical data generated. (Include minimum detection levels and minimum quantification levels.)
- Raw data and bench sheets.
- Provide a description of dechlorination procedures (as applicable).

• Any other observations or test conditions affecting test outcome.

### ATTACHMENT C2

# MARINE CHRONIC TOXICITY TEST PROCEDURE AND PROTOCOL

# I. GENERAL REQUIREMENTS

The permittee shall conduct acceptable silverside chronic (and modified acute) and sea urchin chronic toxicity tests in accordance with the appropriate test protocols described below:

- Inland Silverside (Menidia beryllina) Larval Growth and Survival Test.
- Sea Urchin (Arbacia punctulata) 1 Hour Fertilization Test.

Chronic toxicity data shall be reported as outlined in Section VIII. The chronic <u>Menidia</u> test can be used to calculate an LC50 at the end of 48 hours of exposure when both an acute (LC50) and a chronic (C-NOEC) test is specified in the permit.

# II. METHODS

Methods to follow are those recommended by EPA in:

Klemm, D.J. et al. <u>Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters To Marine and Estuarine Organisms</u>, Second Edition. Environmental Monitoring Systems Laboratory, U.S. Environmental Protection Agency, July 1994, EPA/600/4-91/003.

Any exceptions are stated herein.

# III. SAMPLE COLLECTION

For each sampling event involving the Menidia beryllina, three discharge samples shall be collected. Fresh samples are necessary for Days 1, 3, and 5 (see Section V. for holding times). A single sample is necessary for the Arbacia punctulata test. The sample shall be analyzed chemically (see Section VI). The initial sample (Day 1) is used to start the tests, and for test solution renewal on Day 2. The second sample is collected for use at the start of Day 3, and for renewal on Day 4. The third sample is used on Days 5, 6, and 7. The initial (Day 1) sample will be analyzed chemically (see Section VI). Day 3 and 5 renewal samples will be held until test completion. If either the Day 3 or 5 renewal sample is of sufficient potency to cause lethality to 50 percent or more test organisms in any of the dilutions for either species, then a chemical analysis shall be performed on the appropriate sample(s) as well. Aliquots shall be split from the sample, containerized and preserved (as per 40 CFR Part 136) for the chemical and physical analyses. The remaining sample shall be dechlorinated (if detected) in the laboratory using sodium thiosulfate for subsequent toxicity testing. (Note that EPA approved test methods

require that samples collected for metals analyses be preserved immediately after collection.) Grab samples must be used for pH, temperature, and total residual oxidants (as per 40 CFR Part 122.21).

<u>Standard Methods for the Examination of Water and Wastewater</u> describes dechlorination of samples (APHA, 1992). Dechlorination can be achieved using a ratio of 6.7 mg/L anhydrous sodium thiosulfate to reduce 1 mg/L chlorine. A thiosulfate control (maximum amount of thiosulfate in lab control or receiving water) should also be run.

All samples held overnight shall be refrigerated at 4°C.

# IV. DILUTION WATER

Grab samples of receiving water used for chronic toxicity testing shall be collected from one or several distances away from the discharge. It may be necessary to test receiving water at several distances in a separate chronic test to determine the extent of the zone of toxicity. Avoid collecting near areas of obvious road or agricultural runoff, storm sewers or other point source discharges. An additional control (0% effluent) of a standard laboratory water of known quality shall also be tested.

If the receiving water diluent is found to be, or suspected to be toxic or unreliable, an alternate standard dilution water of known quality with a conductivity, salinity, total suspended solids, organic carbon, and pH similar to that of the receiving water may be substituted **AFTER RECEIVING WRITTEN APPROVAL FROM THE PERMIT ISSUING AGENCY(S)**. Written requests for use of an alternative dilution water should be mailed with supporting documentation to the following address:

Director
Office of Ecosystem Protection
U.S. Environmental Protection Agency, Region 1
One Congress Street
Suite 1100 (CAA)
Boston, MA 02114-2023

It may prove beneficial to the permittee to have the proposed dilution water source screened for suitability prior to toxicity testing. EPA strongly urges that screening be done prior to set up of a full definitive toxicity test any time there is question about the dilution water's ability to support acceptable performance as outlined in the 'test acceptability' section of the protocol.

# V. TEST CONDITIONS AND TEST ACCEPTABILITY CRITERIA

EPA New England requires that tests be performed using <u>four</u> replicates of each control and effluent concentration because the on-parametric statistical tests cannot be used with data from fewer replicates. Also, if a reference toxicant test was being performed concurrently with an effluent or receiving water test and fails, both tests must be repeated.

The following tables summarize the accepted  $\underline{\text{Menidia}}$  and  $\underline{\text{Arbacia}}$  toxicity test conditions and test acceptability criteria:

# EPA NEW ENGLAND RECOMMENDED TEST CONDITIONS FOR THE SEA URCHIN, <u>ARBACIA PUNCTULATA</u>, FERTILIZATION TEST $^{\rm 1}$

Static, non-renewal
30 o/oo ± 2 o/oo by adding dry ocean salts
20 <u>+</u> 1°C
Ambient laboratory light during test preparation
10-20 uE/m²/s, or 50-100 ft-c (Ambient Laboratory Levels)
Disposal (glass) liquid scintillation vials (20 ml capacity), presoaked in control water
5 ml
Pooled sperm from four males and pooled eggs from four females are used per test
About 2000 eggs per chamber and 5,000,000 sperm cells per vial
4 per treatment
Uncontaminated source of natural seawater or deionized water mixed with artificial sea salts
Approximately 0.5
1 hour and 20 minutes
Fertilization of sea urchin eggs
5 and a control. An additional dilution at the permitted effluent concentration (% effluent) is required.

16. Acceptability of test	Minimum of 70% fertilization in controls. E	Effluent
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concentrations exhibiting greater than 70% fertilization, flagged as statistically significantly different from the controls, will not be considered statistically different from the controls for NOEC

reporting.

17. Sampling requirements For on-site tests, samples are to be used within 24

hours of the time that they are removed from the sampling device. For off-site tests, samples must be

first used within 36 hours of collection.

18. Sample volume required Minimum 1 liter

### Footnotes:

Adapted from EPA/600/4-91/003, July 1994.

# EPA NEW ENGLAND RECOMMENDED TEST CONDITIONS FOR THE INLAND SILVERSIDE, MENIDIA BERYLLINA, GROWTH AND SURVIVAL TEST<sup>1</sup>

SILVERSIDE, MENIDIA BERYLLINA, GROWTH AND SURVIVAL TEST<sup>1</sup> 1. Test type Static, renewal 5 o/oo to 32 o/oo + 2 o/oo by adding artificial sea 2. Salinity salts  $25 \pm 1^{\circ}C$ 3. Temperature 4. Light quality Ambient laboratory light  $10-20 \text{ uE/m}^2/\text{s}$ , or 50-100 ft-C5. Light intensity (Ambient Laboratory Levels) 6. Photoperiod 16 hr light, 8 hr darkness 600 - 1000 mL beakers or equivalent (glass test 7. Test vessel size chambers should be used) 8. Test solution volume 500-750 mL/replicate loading and DO restrictions must be met)

9. Renewal of test solutions Daily using most recently collected sample.

10. Age of test organisms Seven to eleven days post hatch; 24 hr range in age.

11. Larvae/test chamber 15 (minimum of 10)

When receiving water is used for dilution, an additional control made up of standard laboratory dilution water (0% effluent) is required.

12. Number of replicate chambers 4 per treatment

13. Source of food Newly hatched and rinsed Artemia nauplii less than

24 hr old

14. Feeding regime Feed once a day 0.10 g wet wt

Artemia nauplii per replicate on days 0-2

feed 0.15 g wet wt Artemia nauplii per replicate on

days 3-6

15. Cleaning Siphon daily, immediately before test solution

renewal and feeding

16. Aeration<sup>2</sup> None

17. Dilution water Uncontaminated source of natural seawater; or

deionized water mixed with artificial sea salts.

18. Effluent concentrations<sup>3</sup> 5 and a control. An additional dilution at the

permitted effluent concentration (% effluent) is

required.

19. Dilution factor  $\geq 0.5$ 

20. Test duration 7 days

21. Effects measured Survival and growth (weight)

22. Acceptability of test

The average survival of control larvae is a

minimum of 80%, and the average dry wt of

unpreserved control larvae is a minimum of 0.5 mg, or the average dry wt of preserved control larvae is a minimum of 0.43 mg if preserved not more than 7

days in 4% formalin or 70% ethanol.

23. Sampling requirements For on-site tests, samples are collected daily and

used within 24 hours of the time they are removed from the sampling device. For off-site tests, sam-

ples must be first used within 36 hours of

collection.

24. Sample Volume Required Minimum of 6 liters/day.

# Footnotes:

Adapted from EPA/600/4-91/003, July 1994.

When receiving water is used for dilution, an additional control made up of standard

If dissolved oxygen (D.O.) falls below 4.0 mg/L, aerate all chambers at a rate of less than 100 bubbles/min. Routine D.O. checks are recommended.

laboratory dilution water (0% effluent) is required.

# VI. CHEMICAL ANALYSIS

As part of each daily renewal of the <u>Menidia</u> test, pH, dissolved oxygen, salinity, and temperature must be measured at the beginning and end of each 24 hour period in each dilution and in the controls. It must also be done at the start of the <u>Arbacia</u> test. The following chemical analyses shall be performed for each sampling event.

			<u>Minimum</u>
			Quantificatio
			<u>n Level</u>
<u>Parameter</u>	<u>Effluent</u>	<u>Diluent</u>	(mg/L)
pH	X	X	
Salinity	X	X	PPT(o/oo)
Total Residual Oxidants *1	X	X	0.05
Total Solids and Suspended Solids	X	X	
Ammonia	X	X	0.1
Total Organic Carbon	X	X	0.5
T-4-1M-4-1-			
Total Metals			
Cd	X	X	0.001
Cr	X	X	0.005
Pb	X	X	0.005
Cu	X	X	0.0025
Zn	X	X	0.0025
Ni	X	X	0.004
Al	X	X	0.02

# **Superscripts:**

Either of the following methods from the 18th Edition of the APHA (1992) <u>Standard Methods for the Examination of Water and Wastewater</u> must be used for these analyses:

- -Method 4500-CL E the Amperometric Titration Method (the preferred method);
- -Method 4500-CL G the DPD Photometric Method.

or use USEPA Manual of Methods Analysis of Water or Wastes, Method 330.5.

# VII. TOXICITY TEST DATA ANALYSIS

LC50 Median Lethal Concentration (Determined at 48 Hours)

Methods of Estimation:

• Probit Method

<sup>\*1</sup> Total Residual Oxidants

- Spearman-Karber
- Trimmed Spearman-Karber
- Graphical

See flow chart on page 56 of EPA/600/4-91/003 for appropriate point estimation method to use on a given data set.

# Chronic No Observed Effect Concentration (C-NOEC)

Methods of Estimation:

- Dunnett's Procedure
- Bonferroni's T-Test
- Steel's Many-One Rank Test
- Wilcoxin Rank Sum Test

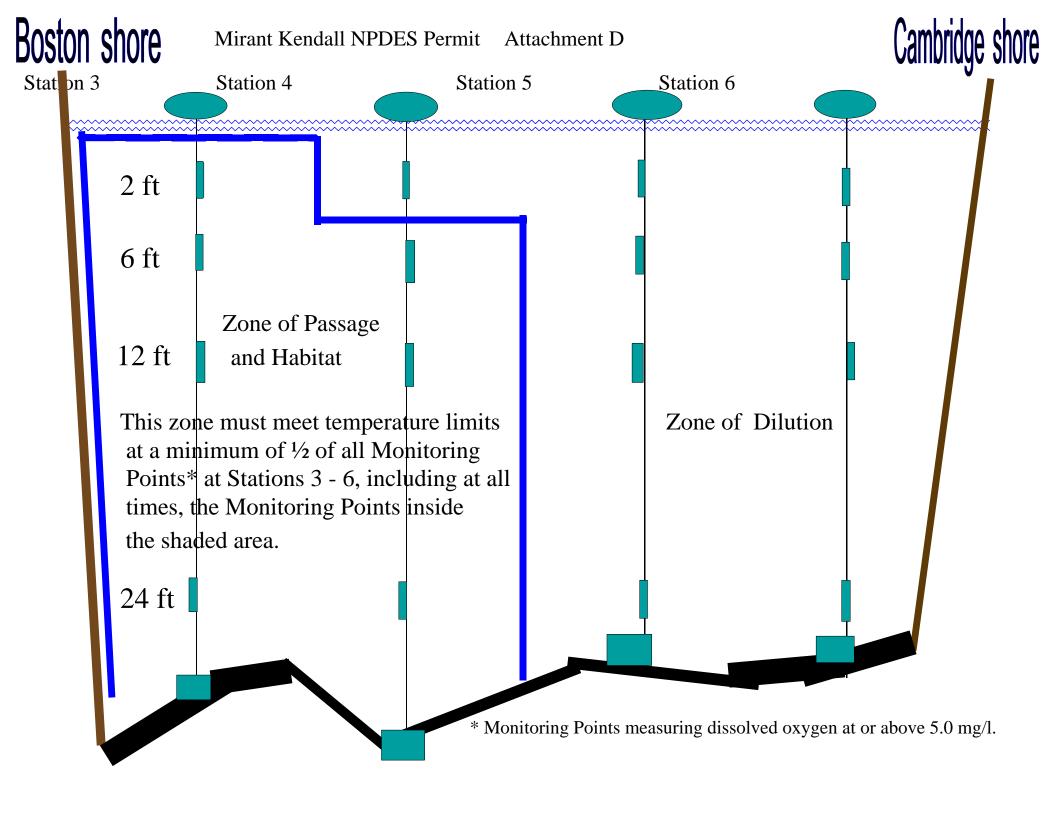
Reference flow charts on pages 191, 192, and 321 of EPA/600/4-91/003 for the appropriate method to use on a given data set.

In the case of two tested concentrations causing adverse effects but an intermediate concentration not causing a statistically significant effect, report the C-NOEC as the lowest concentration where there is no observable effect. The definition of NOEC in the EPA Technical Support Document only applies to linear dose-response data.

# VIII. TOXICITY TEST REPORTING

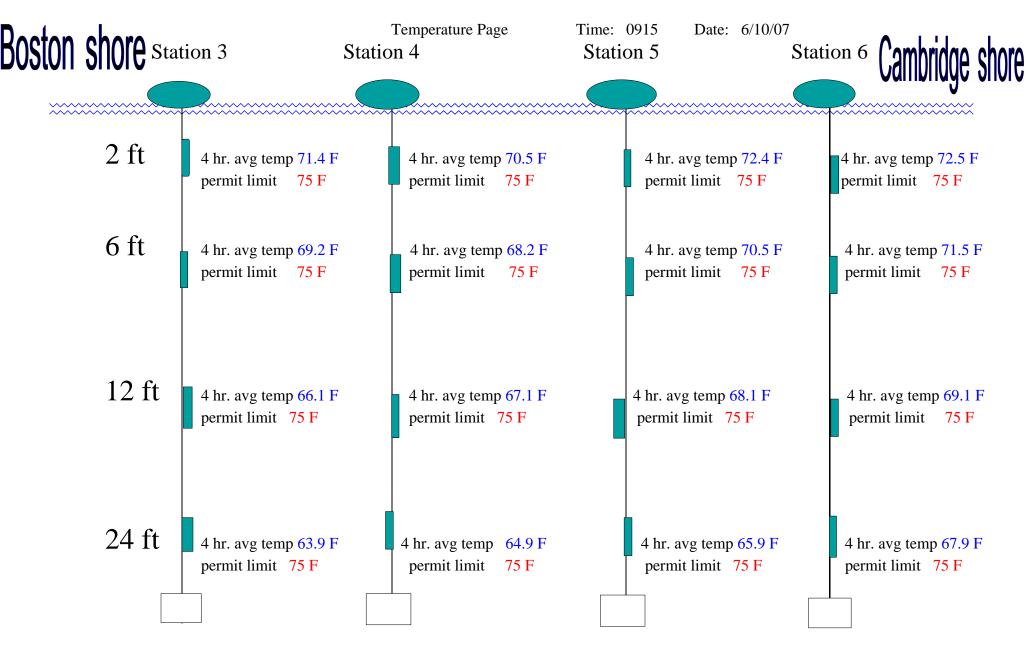
A report of results will include the following:

- Description of sample collection procedures, site description;
- Names of individuals collecting and transporting samples, times and dates of sample collection and analysis on chain-of-custody; and
- General description of tests: age of test organisms, origin, dates and results of standard toxicant tests; light and temperature regime; other information on test conditions if different than procedures recommended. Reference toxicant test data should be included.
- All chemical/physical data generated. (Include minimum detection levels and minimum quantification levels.)
- Raw data and bench sheets.
- Provide a description of dechlorination procedures (as applicable).
- Any other observations or test conditions affecting test outcome.



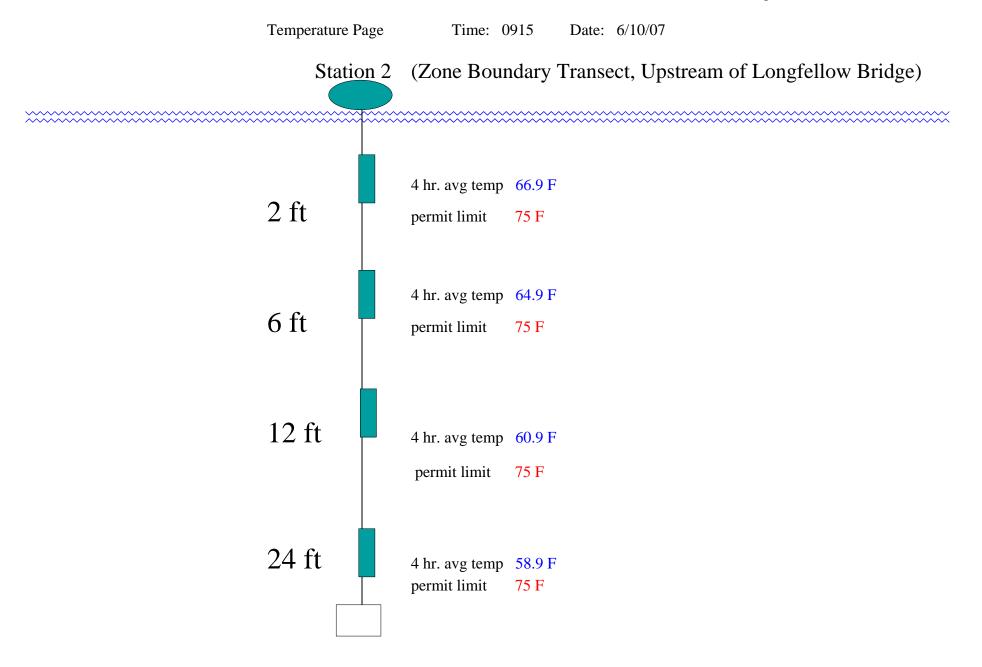


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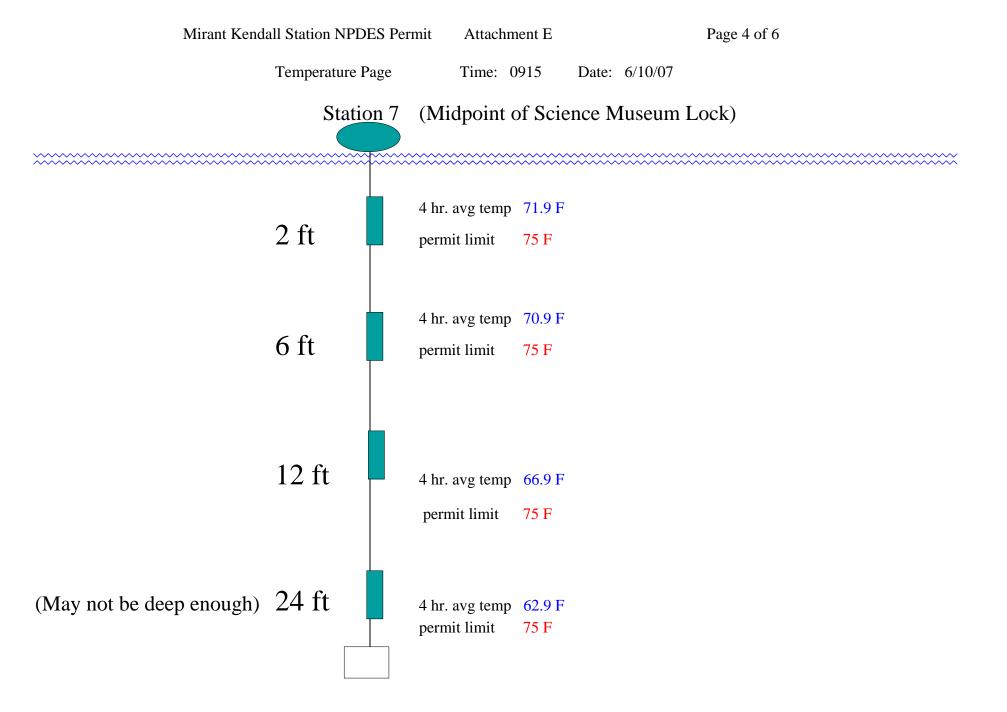


Over 50% Permit Limits Exceeded: NO Current Number of Exceedences over 50%: 0 Number of Exceedences over 50% in Past 7 Days: 0 Limits Met At All Points of Station 3: YES Limits Met At All Points of Station 4 (except 2 ft): YES

Note: A similar information page must be created for each Monitoring Station for dissolved oxygen values, if real-time continuous DO readings are required.



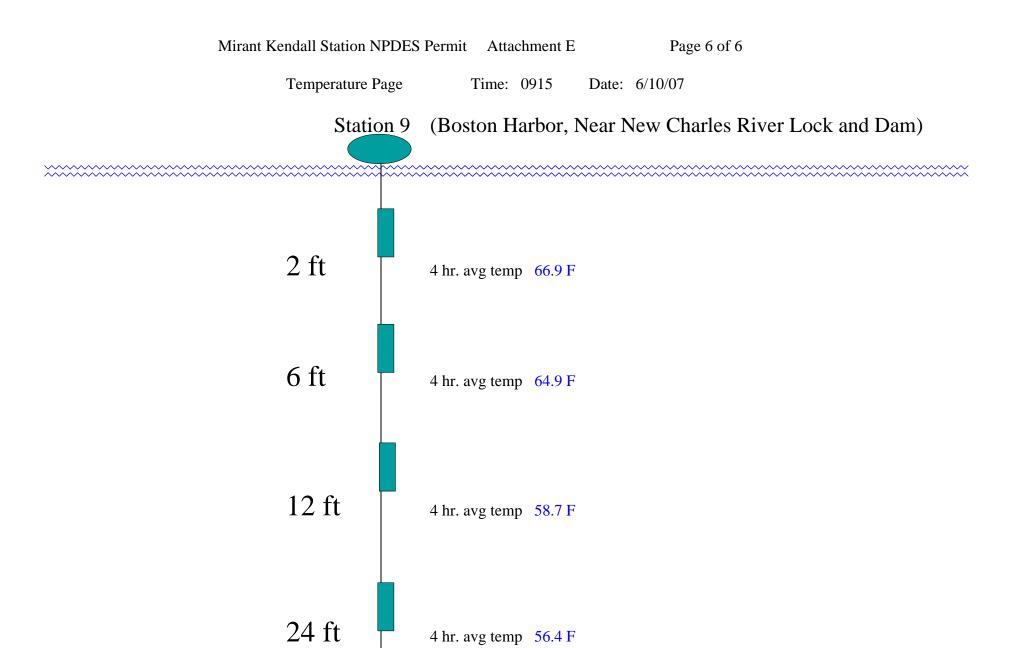
Limits Met At All Points of Station 2: YES Number of Exceedances in Past 7 Days: 0



Limits Met At Two Contiguous Points of Station 7: YES Number of Exceedances in Past 7 Days: 0

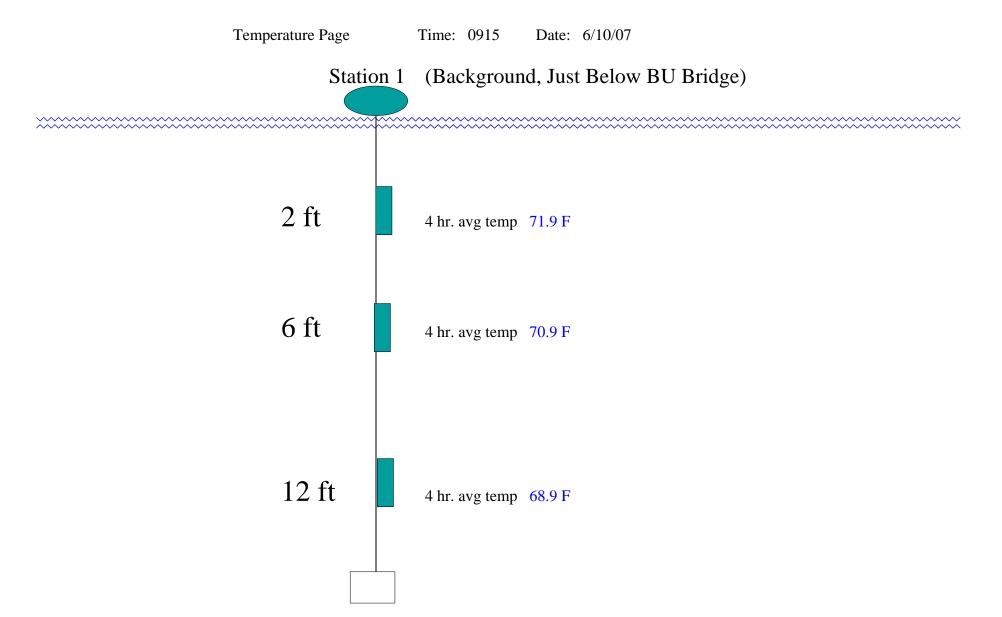


Limits Met At All Points of Station 8: YES Number of Exceedances in Past 7 Days: 0



Monitor and Report Only

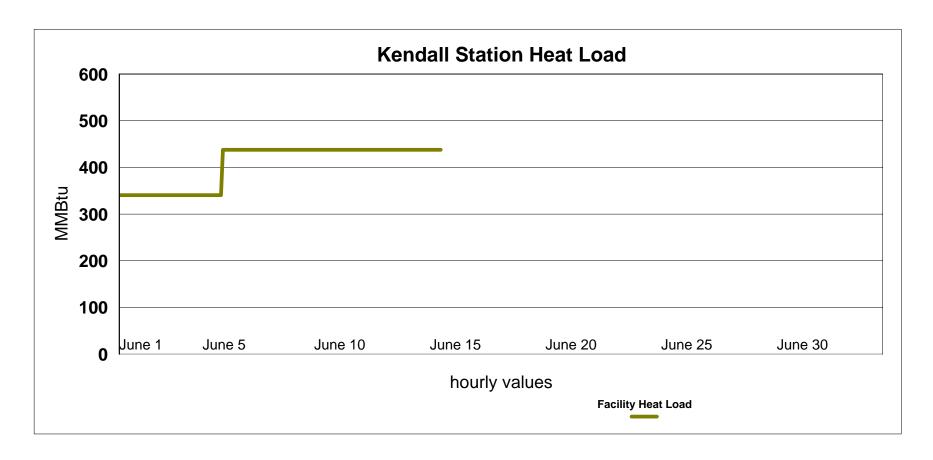
No Limits In Effect For Station 9



Used To Determine Ambient River Conditions Only

No Limits In Effect For Station 1

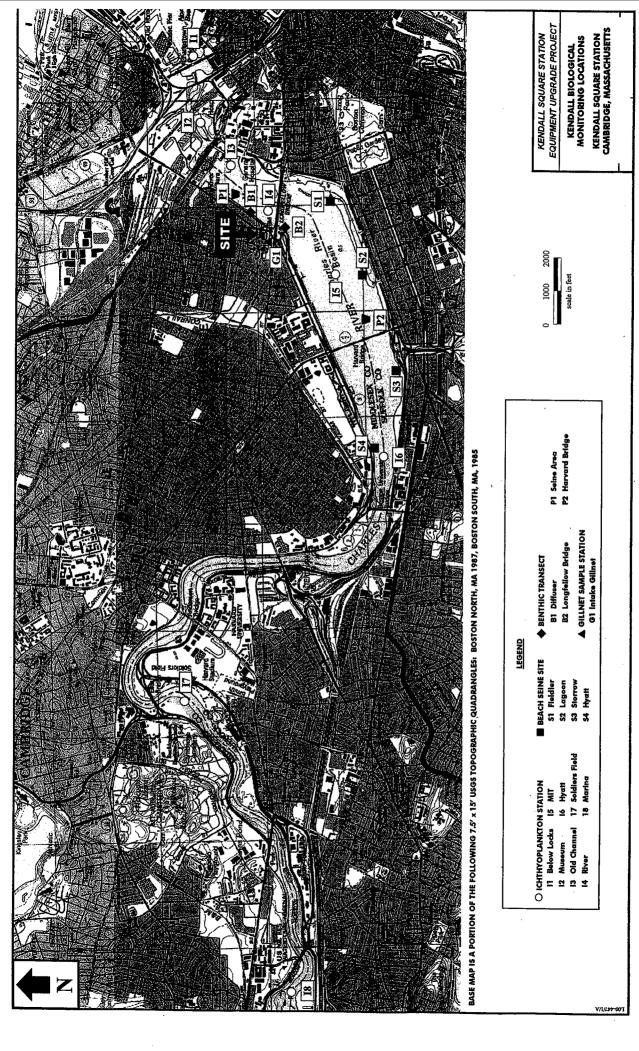
An example of hourly heat load values updated in real-time depicting the heat load at Kendall Station displayed continuously and in real-time on a web page that can be accessed by EPA and MassDEP. NOTE: Meteorological parameters listed in the permit must also be displayed in a similar format.



# Kendall Station Supplemental Monthly Report Form A Maximum Temperature Limit Violation In The Zone Of Passage and Habitat

Date	Station Number	Depth	Number of 4 Hour Time Blocks In Violation	Maximum Temperature Reached	Units deg F	Temperature Limit In Effect	Units deg F	From April 15 Through June 7 Only If Used As One Of Six Temperature Excursions, Record Total Used To Date Including This Excursion (enter number 1 through 6, if applicable)

# ATTACHMENT H



Attachment I - Water Quality and Biological Monitoring Requirements \*

<b>Monitoring Activity</b>	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	ОСТ	NOV	DEC	Permit Part I.A
Water quality (WQ) continuous	С	С	С	С	С	С	С	С	С	С	С	С	14.b
WQ - contour mapping <sup>1</sup>	M	M	M	M	M	M	M	M	M	M	M	M	14.c
WQ - nutrients						W	W	W	W	W			14.c.2
WQ - continuous plant data	С	С	С	С	С	С	С	С	C	С	С	С	14.b.9
Meteorological Data	С	С	С	С	С	С	С	С	С	С	С	С	14.b.10
Phytoplankton - sample collection						W	W	W	W	W			14.d.10
Ichthyoplankton - river samples			W	W	W	W	W	W					14.d.4
Ichthyoplankton - barrier study <sup>2</sup>					V	V	V						14.d.7
Fish - beach seine collection							W	W	W	W	W		14.d.1
Fish - pushnet collection						W	W	W	W	W			14.d.1
Fish - gill/fyke net collection <sup>3</sup>			W	W	W	W	W	W	W	W	W		14.d.2
Fish - plant impingement	W	W	W	W	W	W	W	W	W	W	W	W	14.d.9
Fish - anadromous fish counts				3W	3W	3W							14.d.5
Fish - fish tagging/movement				С	С	С							14.d.6
Fish - outfall pipe monitoring				W	W	W							14.d.8

<sup>\*</sup> For summary purposes only C = continuous; W = weekly; 3W = three times per week; M = monthly; V = variable

- 1. Monthly for the first 2 years, then quarterly thereafter
- 2. One year only
- 3. After the first year, shall be monitored once every two weeks.