QUALITY ASSURANCE PROJECT PLAN

Wet Weather Sampling of Mt. Hope Bay and Kickemuit River

Rhode Island Department of Environmental Management
Office of Water Resources

March 17, 2006

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Table 3.1 QAPP Distribution List.

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3.1 Acronym List

RIDEM: The Rhode Island Department of Environmental Management

USEPA: The United States Environmental Protection Agency

MADMF: The Massachusetts Department of Marine Fisheries

RIDOH: The Rhode Island Department of Health

4.0 Project Organization

4.1 Project Organizational Chart

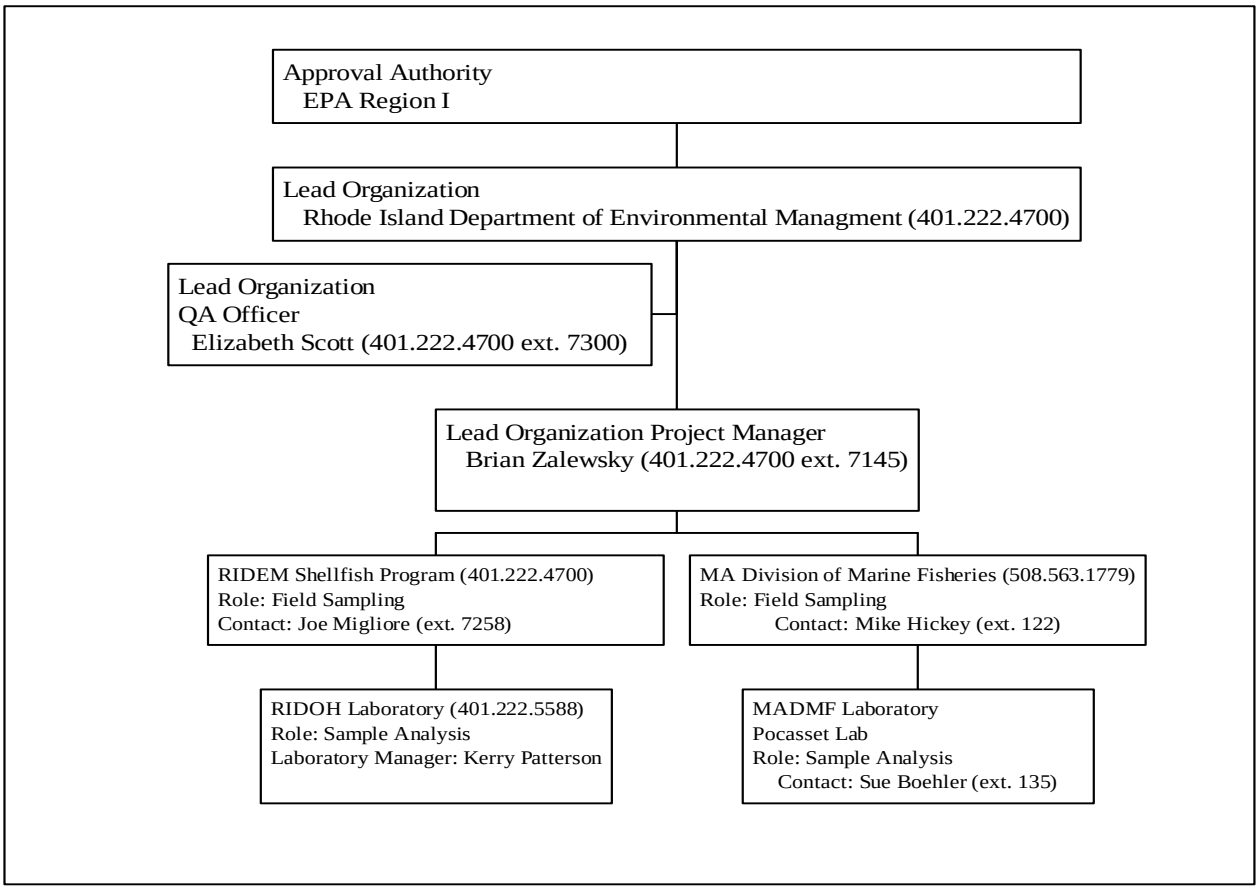


Figure 4.1 Project Organizational Chart.

4.2 Communication Pathways

The sampling plan details sample collection in estuarine waters as well as source sampling in the Rhode Island waters of Mount Hope Bay and the Kickemuit River estuary. Sampling teams will be comprised of staff from RIDEM and MADMF Shellfish Unit. The Rhode Island Department of Health Laboratory (RIDHL) will analyze all estuarine samples collected by RIDEM staff for fecal coliform. Point source samples collected by RIDEM staff will be analyzed by the RIDHL for both fecal coliform and male-specific bacteriophage. RIDEM is coordinating with the Massachusetts Division of Marine Fisheries (MADMF) Shellfish Unit in conducting a companion study of estuarine waters in the Massachusetts portion of Mount Hope Bay waters and in the Lee, Cole, and Taunton Rivers. The Massachusetts Division of Marine Fisheries (MADMF) Laboratory in Pocasset, MA will analyze all estuarine samples collected by MADMF staff. Split samples will be collected by MADMF at five estuarine sampling stations in the Massachusetts portion of Mount Hope Bay for analysis at RIDHL. Data collected by the MADMF Shellfish Program will be kept separate from RIDEM collected data but can be accessed by contacting Susan Boehler, Lab Supervisor at 508.563.1779.

The Project Manager will contact RIDHL to arrange for sample bottles prior to sampling. Sample bottles will be kept at RIDEM and will be used for the pre-storm sampling. When a potential storm is forecast, the Project Manager will alert the RIDHL to arrange for the pickup of additional bottles. Brian Zalewsky of RIDEM will coordinate the arrangements with the RIDHL.

Changes to the sampling plan may occur during the course of the wet weather surveys. All changes made in the field by the field samplers will be documented in the field notes. The Project Manager will discuss these changes with the field samplers within three days after sampling. It may become necessary to add and drop stations prior to sampling. This decision will be made jointly by the QA Officer and the Project Manager. All changes to the QA Plan will be reported in each event's Status Report and the Final Report.

4.3 Training

Each sampler will be given a copy of the Quality Assurance Plan that outlines the station locations and sampling protocol before sampling begins. All samplers will also be given a tour of their sampling locations prior to the first wet weather event. The Project Manager will detail the protocol for each station during the sampling station tour and distribute maps with station locations. All marine stations are well documented and shellfish staff, who routinely sample this area, will be sampling these locations. Source stations will be sampled by DEM staff who are familiar with bacteria sampling techniques and knowledgeable with the area.

5.0 Introduction

Mount Hope Bay and the Kickemuit River are on Rhode Island's 2004 303(d) list of impaired waterbodies due to violations of one or more water quality criteria. RIDEM is currently developing Total Maximum Daily Loads (TMDLs) to address pathogen impairments in these waterbodies. The TMDL study will begin in Spring 2006 and will include wet weather bacteria sampling at approximately thirty-seven (37) stations within both waterbodies. Analysis of available data indicates that bacteria concentrations in Mt. Hope Bay and the Kickemuit River increase significantly during wet weather events. The primary sources appear to be stormwater and CSO discharges, however the available wet weather dataset is over 15 years old and significant improvements to the CSO system have recently been made by the city of Fall River, MA. In RI, the study area waters are operated as a conditional shellfish growing area with closures put in effect for a 7-day period following a rain event of 0.5 inches or more. Consistent with FDA requirements, RIDEM monitors water quality conditions when the area is open to shellfishing (i.e. 12 times per year during dry weather conditions).

RIDEM is proposing the plan outlined in this QAPP to conduct a field measurement program to characterize bacteria conditions at existing shellfish sampling stations in Mt. Hope Bay and the Kickemuit River during periods of wet weather. Wet weather data will be used in conjunction with existing data to develop fecal coliform TMDLs for waterbodies within Mt. Hope Bay and the Kickemuit River. Staff from the Massachusetts Department of Marine Fisheries (MADMF) Shellfish Sanitation and Management Program will be sampling growing areas in Mount Hope Bay, Lee River, Coles River, and Taunton River, including those adjacent to RI growing area waters.

5.1 Study Area

Mount Hope Bay forms the northeast corner of the Narragansett Bay estuary and covers an area of about 36 km^2 (Figure 5.1). The Rhode Island-Massachusetts State boundary traverses the area in a southeasterly direction. Although over 70% of Mt. Hope Bay is located in Rhode Island, over 90% of its drainage basin is located in Massachusetts. The drainage area covers more than 1476 km^2. Major rivers discharging into the Bay include the Taunton, Cole, Lee, Kickemuit, and Quequechan. Each of these rivers originates and terminates in Massachusetts, with the exception of the Kickemuit, which terminates in Rhode Island waters. The Taunton River is by far the largest freshwater source with a mean daily flow rate of approximately 18 m^3/sec at its mouth.

Mt. Hope Bay empties into the East Passage of Narragansett Bay and the Sakonnet River. Approximately 70% of the Bay has a mean low water depth of 5.5 meters or less. The mean tide range is approximately 1.4 meters. The average currents are approximately 0.4 and 0.5 knots on the flood and ebb tides, respectively (ASA 1990). Wind direction strongly influences the Bay water's mixing patterns, with the greatest mixing provided by southerly winds (ASA 1990).

The Kickemuit River forms the most northwestern embayment of the Mt. Hope Bay estuary, extending northwest from its mouth at Mt. Hope Bay into portions of the Towns of Swansea and Rehoboth, MA. The Kickemuit River originates in Rehoboth and flows into the Warren reservoir in northern Swansea. From here, the river flows south under interstate 195 and then Rt. 6 toward the MA – RI border where it empties into the Kickemuit Reservoir. The dam at the southern end of the Kickemuit Reservoir marks the boundary between the river's fresh and salt-water segments. Through the remainder of the town of Warren and the northeastern portion of Bristol, the river is tidal.

Table 5.1 and Figure 5.1 describe and present the applicable waterbodies (i.e. the study area) within the Kickemuit River and Mount Hope Bay sub basin. The TMDL study area encompasses all waters within RIDEM-classified growing areas (GA) 5 and 17.

Table 5.1. Applicable waterbodies within the study area.

Waterbody ID Number	Waterbody Description	Classification
RI0007032E-01D	Mt. Hope Bay waters south and west of the MA-RI border and north of a line from Borden's Wharf, Tiverton to buoy R "4" and east of a line from buoy R "4" to Brayton Point in Somerset, MA. Bristol, Portsmouth, and Tiverton	SB1
RI0007032E-01C	Mt. Hope Bay waters south of a line from Borden's Wharf, Tiverton, to buoy R "4" and west of a line from buoy R "4" to Brayton Point, Somerset, MA, and east of a line from the end of Gardiner's Neck Road in Swansea to buoy N "2" through buoy C "3" to Common Fence Point, Portsmouth, and north of a line from Portsmouth to Tiverton at the railroad bridge at "The Hummocks" on the northeast point of Portsmouth.	SB
RI0007032E-01A	Mt. Hope Bay south and west of the MA/RI border, and east of a line from Touisset Point to the channel marker buoy R "4" and south and east of a line from buoy R "4" to the southernmost landward end of Bristol Point and south of a line from Bristol Point to the Hog Island shoal light to the southwestern extremity of Arnold Point in Portsmouth where a RIDEM range marker has been established; And west of a line form the end of Gardiner's Neck Road, Swansea to buoy N "2", through buoy C "3" to Common Fence Point, Portsmouth excluding the waters defined in RI0007032E-01E below. Warren, Portsmouth	SA
RI0007032E-01B	Mt. Hope Bay waters north and west of a line from the southernmost landward end of Bristol Point to buoy R "4" and west of a line from buoy R "4" to the DEM range marker on Touisset Point, and south of the Bristol Narrows. Bristol, Warren	SA
RI0007033E-01A	Kickemuit River from the Child Street bridge (Route 103) in Warren, south to the river mouth at "Bristol Narrows" excluding the waters described below. Bristol, Warren	SA
RI0007033E-01B	Kickemuit River south of a line from the eastern extension of Kickemuit Avenue in Bristol to the DEM range marker located on the western tip of Little Neck in Touisset, and north of a line from the DEM range markers located on the east shore and west shore at the entrance to the Kickemuit River including the "Bristol Narrows" in its entirety. Bristol, Warren	SAb
RI0007033E-01C	Kickemuit River west of a line from the DEM range marker located on the western tip of Little Neck in Touisset to the brick stack located at 426 Metacom Avenue in Warren (formally known as the Carol Cable Building), north of a line from the eastern extension of Sherman Avenue in Bristol to the western extension of Chase Avenue Touisset, and south of a line from the eastern extension of Harris Avenue in Warren to the "5MPH No Wake" buoy. Bristol, Warren.	SAb

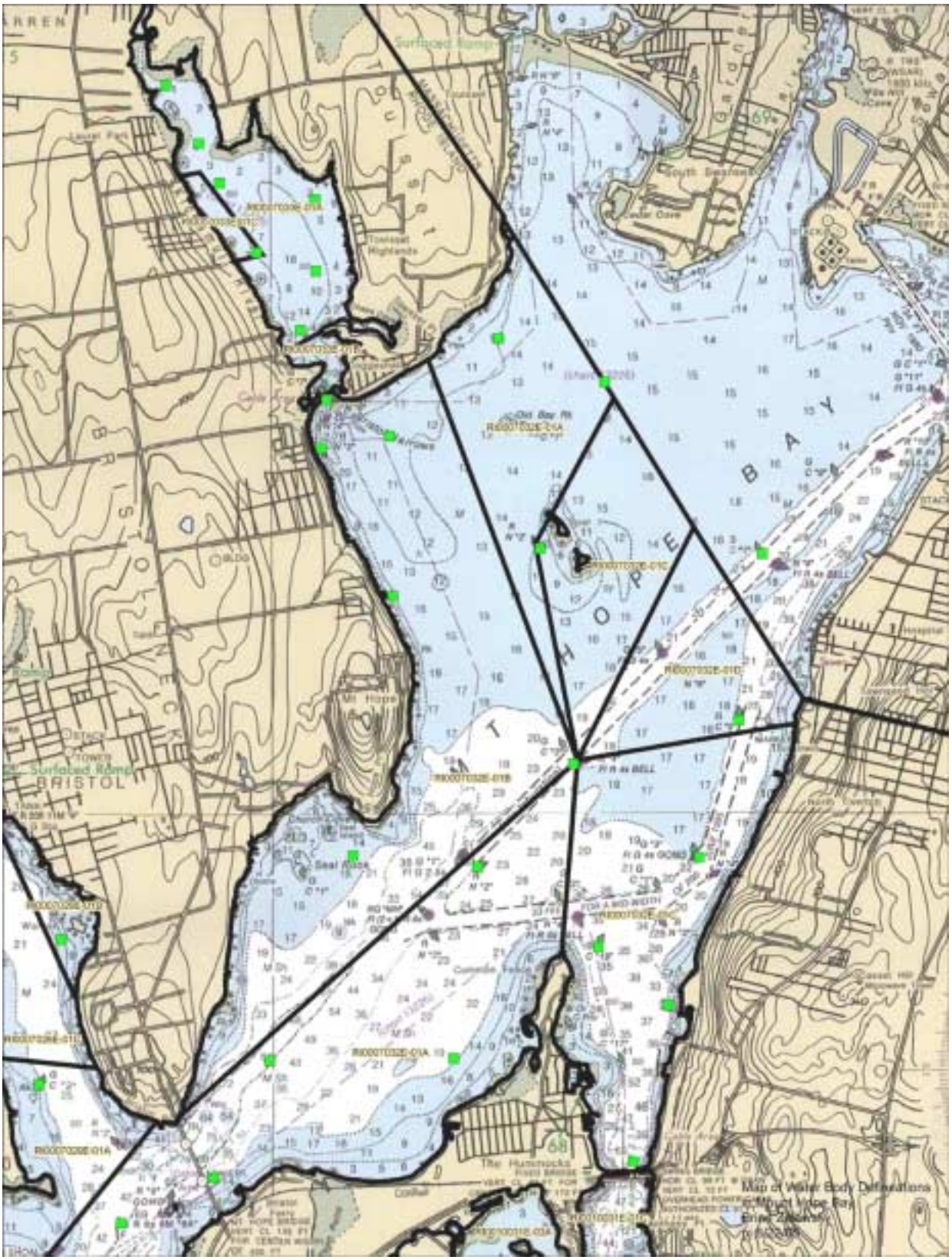


Figure 5.1. General site map of Mount Hope Bay and Kickemuit River. RIDEM GA5 and GA17 sampling stations highlighted in green

5.2 Applicable Water Quality Standards

SA, SB, and partial use waters exist within the study area. Designated uses for these waters are described in Rhode Island's Water Quality Regulations, as follows:

Class SA waters are designated for shellfish harvesting for direct human consumption, primary and secondary contact recreational activities, and fish and wildlife habitat.

Class SAb waters are in the vicinity of marinas and/or mooring fields and therefore seasonal shellfishing closures will likely be required, however all Class SA criteria must be attained.

Class SB waters are designated for primary and secondary contact recreational activities; shellfish harvesting for controlled relay and depuration, and fish and wildlife habitat.

Class SB1 waters are designated for primary and secondary contact recreational activities and fish and wildlife habitat. Primary contact recreational activities may be impacted due to pathogens from approved wastewater discharges. However all Class SB criteria must be met.

The fecal coliform standards for Class SA, SB, partial use waters are established in Rule 8.D. of DEM's Water Quality Regulations. Applicable water quality standards in the study area are designed to protect designated uses of shellfishing and/or primary contact recreation/swimming. Water Quality Regulations require all SA and SA partial use waters to meet shellfishing criteria. All SB and SB partial use waters must meet primary contact recreational/swimming criteria. The shellfishing standard specifies that the maximum allowable level of fecal coliform bacteria (Most Probable Number (MPN) per 100 milliliter) may not exceed a geometric mean MPN value of 14 and not more than 10% of the samples shall exceed an MPN value of 49.

The primary contact recreational/swimming criteria, which applies to all Class SA and SB waters, specifies that the maximum allowable level of fecal coliform bacteria may not exceed a geometric mean value of 50 MPN/100ml and not more than 10% of the total samples taken shall exceed 400 MPN/100ml.

Designated shellfish waters within the study area are classified as "Permanently Closed", "Conditional", or "Seasonally Approved" (Figures 5.2 and 5.3). Regardless of classification, all areas are currently sampled 12 times per year when the waters are open. Ten (10) stations are sampled in GA5 and 16 stations are sampled in GA17. All sampling for bacteriological evaluation of the growing waters is conducted according to a systematic random schedule. This requires that sample collection be scheduled sufficiently far in advance to support random collection with respect to environmental conditions. Currently, all waters within the study area must be closed for seven (7) days following a precipitation event equal to or greater than 0.5 inches (1.3 cm).

Prior to 1996, all waters within GA17 were classified as "Prohibited". In 1995, waters within Mount Hope Bay and the Kickemuit River were re-evaluated by RIDEM. This reevaluation recommended that portions of the area be reclassified from 'restricted' to 'conditionally approved' for the harvesting of shellfish if certain conditions were met.

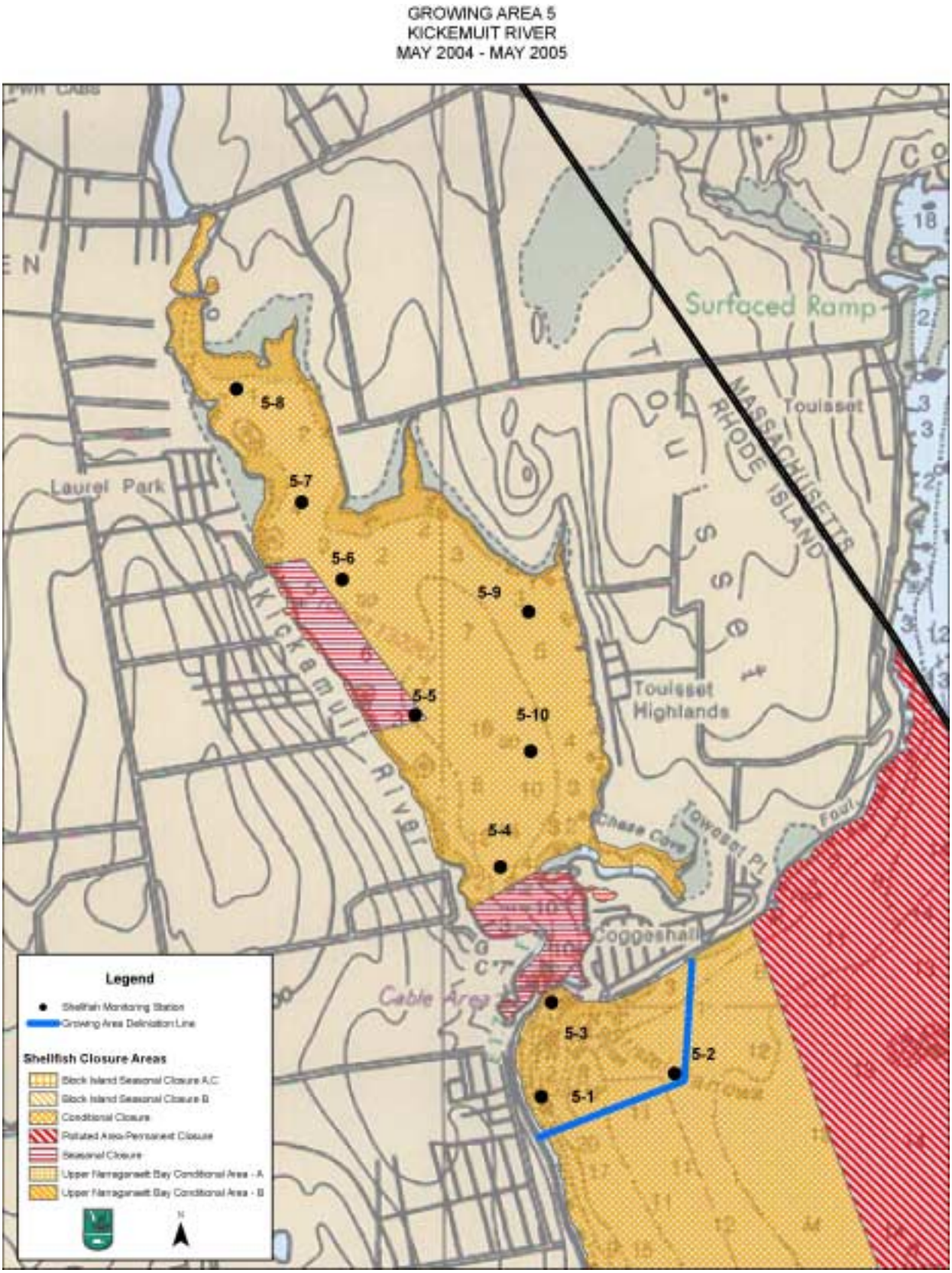


Figure 5.2. Growing Area 5 (Kickemuit River) with sampling station locations and growing area classifications.

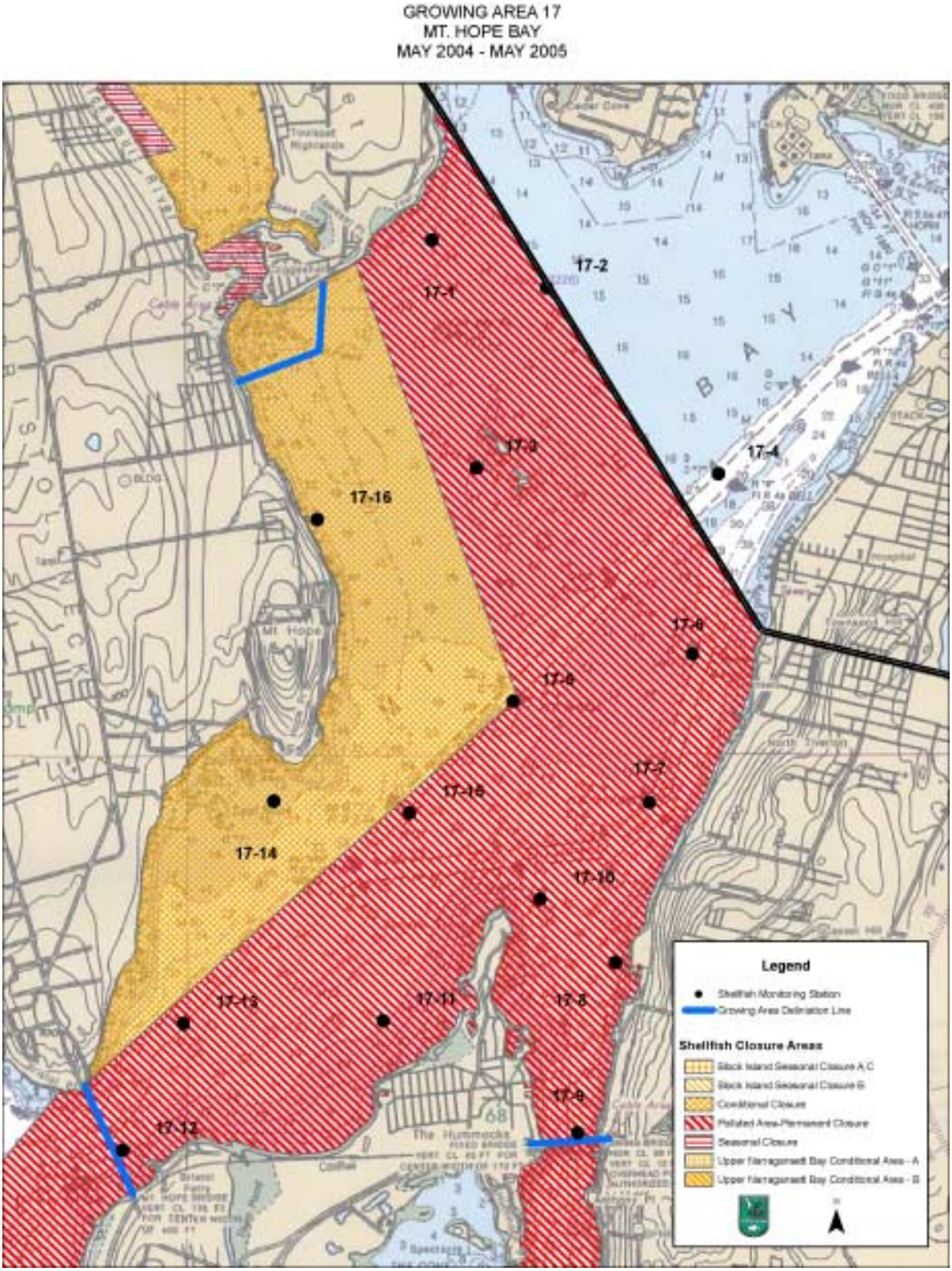


Figure 5.3. Growing Area 17 (Mt. Hope Bay) with sampling station locations and growing area classifications.

5.3 Existing Water Quality Conditions

All shellfish stations within GA5 and GA17 are currently sampled, on a random basis, twelve times per year when the waters are open. An annual statistical analysis of the data is used to determine whether water quality within the growing area meets water quality standards and complies with applicable National Shellfish Sanitation Program (NSSP) requirements. These analyses include the calculation of the geometric mean and 90th percentile of each station's fecal coliform dataset (n=15). The most recent analyses are presented below in Table 5.2. All SA waters must meet a geometric criteria of 14 MPN and a 90th percentile criteria of 49 MPN, while all SB waters must meet a geometric mean criteria of 50 MPN and a 90th percentile criteria of 400 MPN. These criteria apply to shellfishing (SA) and swimming (SB) uses, respectively.

Table 5.2. Most recent statistical summary for GA17 and GA5 (Feb 2006).

Station Name	Status	Class	n	Geometric Mean Value	Geometric Mean Criteria	90th Percentile Value	90th Percentile Criteria
Mt. Hope Bay							
GA17-1	P	SA	15	8.6	14	**51.7**	49
GA17-2	P	SA	15	9.4	14	**49.4**	49
GA17-3	P	SA	15	**150.2**	14	**1865.4**	49
GA17-4	P	na	15	11.1	14	**64.5**	49
GA17-5	P	SA	15	12.5	14	**170.5**	49
GA17-6	P	SB1	15	7.2	50	47.0	400
GA17-7	P	SB	15	6.6	50	32.6	400
GA17-8	P	SB	15	5.5	50	22.2	400
GA17-9	P	SB	15	4.4	50	11.6	400
GA17-10	P	SB	15	5.9	50	31.2	400
GA17-11	P	SB	15	5.3	50	29.0	400
GA17-12	P	SB	15	4.7	50	20.6	400
GA17-13	P	SB	15	4.7	50	26.8	400
GA17-14	CA	SA	15	7.2	14	**53.0**	49
GA17-15	P	SB	15	10.4	50	**70.3**	400
GA17-16	CA	SA	15	6.5	14	38.0	49
Kickemuit River							
GA5-1	CA	SA	15	5.5	14	25.8	49
GA5-2	CA	SA	15	7.3	14	36.5	49
GA5-3	CA	SA	15	5.1	14	25.6	49
GA5-4	CA	SA	15	5.0	14	15.0	49
GA5-5	CA-SA	SAb	15	3.7	14	10.0	49
GA5-6	CA	SA	15	4.4	14	12.8	49
GA5-7	CA	SA	15	4.8	14	16.0	49
GA5-8	CA	SA	15	7.4	14	27.0	49
GA5-9	CA	SA	15	2.8	14	7.7	49
GA5-10	CA	SA	15	3.7	14	13.7	49

Status: P: Prohibited, CA: Conditional Area

Both swimming and shellfishing criteria are met at all stations within GA5 (Kickemuit River). In GA17 (Mt. Hope Bay) only one station, GA17-3 violates the geometric mean portion (14 MPN) of the shellfishing criteria. Stations GA17-1, 2, 3, 4, 5, 14, and 15 violate the shellfishing 90th percentile criteria of 49 MPN. Station GA17-3 violates the 90th percentile portion of the swimming criteria (400 MPN).

A more comprehensive analysis was performed using all available data collected from 1985 to 2005. These data were examined for trends in fecal coliform concentrations under varied weather and tidal conditions. Tables 5.3 and 5.4 present these analyses. From 1984 to 1996, and prior to reclassification, all stations within GA5 and GA17 were randomly sampled 12 times annually during both dry and wet weather. Therefore, wet weather data exist for years 1985-1996, while data from 1996 through the present were collected during dry weather only.

Overall, the data show distinct differences in water quality under dry and wet weather conditions. As expected, both geometric mean and 90th percentile values are highest during wet weather conditions. The

combination of wet weather and flood tide appears to produce the largest concentrations seen in the 1985-1996 dataset. The 90th percentile criteria, not to exceed 49 MPN, is violated at all stations except GA17-8. Station GA17-3, located near Spar Island exhibits the most elevated geometric mean and percentile values of all stations within GA17. The largest wet weather fecal coliform bacteria concentrations measured in GA5 are seen at three stations (GA5-1, 2, and 3), all located at or outside of the mouth of the Kickemuit River.

Table 5.3. Statistical summary of fecal coliform data from 1984-2005 (April 2005) for GA17.

	GA17-1		GA17-2		GA17-3		GA17-4		GA17-5		GA17-6	
Condition	gm	90th	gm	90th	gm	90th	gm	90th	gm	90th	gm	90th
1996-2005 DW ebb	5	16	6	43	24	430	6	88	5	25	5	43
1996-2005 DW flood	4	23	7	28	10	93	6	28	4	23	4	12
1984-1996 Dry	5	23	9	67	15	230	10	84	7	43	8	230
1984-1996 Wet	9	93	16	237	43	240	24	430	11	189	12	203
1984-1996 WW flood	11	93	23	316	25	510	21	430	14	148	14	148
1984-1996 WW ebb	8	61	13	148	73	240	27	290	8	148	11	176
1984-1996 DW flood	6	28	9	48	9	107	7	25	5	43	5	44
1984-1996 DW ebb	4	15	8	68	29	230	18	512	10	237	13	235
	GA17-7		**GA17-8**		**GA17-9**		**GA17-10**		**GA17-11**		**GA17-12**	
Condition	gm	90th	gm	90th	gm	90th	gm	90th	gm	90th	gm	90th
1996-2005 DW ebb	5	41	4	15	4	9	3	9	3	8	3	10
1996-2005 DW flood	4	9	4	15	4	12	4	15	4	15	3	15
1984-1996 Dry	6	43	4	15	5	19	5	13	4	11	3	9
1984-1996 Wet	11	93	8	37	14	189	11	133	8	91	8	116
1984-1996 WW flood	8	43	8	31	22	230	15	118	7	63	7	150
1984-1996 WW ebb	13	134	7	31	9	56	8	116	9	90	8	4
1984-1996 DW flood	5	28	3	10	4	16	5	10	4	15	3	9
1984-1996 DW ebb	6	43	5	19	5	17	4	13	4	43	5	
	GA17-13		**GA17-14**		**GA17-15**		**GA17-16**					
Condition	gm	90th	gm	90th	gm	90th	gm	90th				
1996-2005 DW ebb	4	16	4	16	5	18	2	25				
1996-2005 DW flood	4	18	4	18	4	43	3	9				
1984-1996 Dry	5	31	5	25	7	43	7	93				
1984-1996 Wet	11	82	10	43	13	91	10	139				
1984-1996 WW flood	7	63	9	63	14	230	12	318				
1984-1996 WW ebb	15	77	11	43	13	77	9	58				
1984-1996 DW flood	3	9	4	23	5	43	5	23				
1984-1996 DW ebb	8	43	5	26	9	58	9	93				

Table 5.4. Statistical summary of fecal coliform data from 1984-2005 (April 2005) for GA5.

	GA5-1		GA5-2		GA5-3		GA5-4		GA5-5	
Condition	gm	percentile	gm	percentile	gm	percentile	gm	percentile	gm	percentile
1996-2005 DW ebb	4	9	5	23	4	9	3	9	3	9
1996-2005 DW flood	4	17	4	16	4	23	3	8	3	8
1984-1996 Dry	6	33	6	43	7	9	3	9	3	9
1984-1996 Wet	9	93	12	93	8	189	7	104	7	104
1984-1996 WW flood	25	516	17	530	24	554	14	278	14	278
1984-1996 WW ebb	5	15	10	93	4	16	4	17	4	17
1984-1996 DW flood	6	31	5	27	7	53	3	7	3	7
1984-1996 DW ebb	6	58	7	43	6	43	4	15	4	15
	GA5-6		**GA5-7**		**GA5-8**		**GA5-9**		**GA5-10**	
	gm	percentile	gm	percentile	gm	percentile	gm	percentile	gm	percentile
1996-2005 DW ebb	4	23	4	9	4	9	3	7	3	4
1996-2005 DW flood	3	15	3	9	3	9	3	4	3	6
1984-1996 Dry	3	11	4	13	4	13	4	13	4	15
1984-1996 Wet	6	53	6	27	6	27	8	43	7	57
1984-1996 WW flood	13	278	11	184	11	184	12	342	14	284
1984-1996 WW ebb	4	10	5	17	5	17	5	44	5	23
1984-1996 DW flood	3	6	3	7	3	7	3	5	3	12
1984-1996 DW ebb	3	11	5	43	5	43	5	44	4	23

In 1990, Applied Science Associates, Inc. (ASA) conducted dry and wet weather water quality surveys, including CSO monitoring and dye studies, in Mt. Hope Bay, primarily the Massachusetts portion. The wet weather component consisted of three intensive measurement studies, each spanning a three-day period, beginning shortly before a storm of at least 0.4 inches (1.0 cm) of precipitation. The majority of stations were located within the upper northeast portion of the bay, within Massachusetts.

Results from the ASA surveys showed that fecal coliform levels in the bay were dramatically affected by storm-related runoff. In addition, the study showed that the combined loadings of the CSO's along the bay clearly accounted for the high fecal coliform levels observed in the bay during and shortly thereafter the storm. Typical fecal coliform densities from the CSOs ranged from 10^5 to over 10^6 per 100ml. Mid- and post-storm fecal coliform levels typically exceeded several hundred colonies per 100ml at the stations adjacent to the shoreline of Fall River. Peak values ranging from 220 to 12000 col/100ml were observed in the bay during wet weather studies. For all three wet weather surveys, fecal coliform levels in the bay dropped rapidly once CSO flows ended and dropped to background levels within 24 hours.

Survey data also indicated that the CSO effluent from the city of Fall River discharged to the bay during the storms remained concentrated near the surface, while diffusing slowly downward during the course of each study. Most of the effluent remained near the east shore of the bay, moving slowly toward the Mt. Hope Bridge.

5.4 Actual and Potential Pollution Sources

To date, and largely through the efforts of the RIDEM OWR Shellfish Program, US Food and Drug Administration (FDA), MADEP, and MADMF numerous point and nonpoint sources of fecal contamination affecting the study area have been identified. These currently include, or have included dry and wet weather CSO discharges from the city of Fall River, WWTF discharges, and direct stormwater runoff. Other potential sources in both RI and MA may also include:

- leaking sewer pipes,
- stormwater runoff
- stormwater drainage systems (illicit connections of sanitary sewers to storm drains),
- failing septic systems,
- recreational activities, and
- waterfowl and wildlife
- agricultural runoff

According to a 1988 FDA survey of Mt. Hope Bay (Rippey et al. 1988), point sources of primary concern were the Quequechan River, the Taunton River, several of the combined sewer overflows (CSOs) discharging from Fall River, several stormwater discharges along the western shoreline of the Bay south of the Kickemuit River, and the Somerset WWTF.

The RIDEM OWR Shellfish program conducted dry weather sanitary surveys of Mt. Hope Bay and the Kickemuit River in 2002 to identify pollution sources that have potential to impact these waters during periods when the growing area is open to harvest (i.e. 0.5 inches of rain or less). These data are presented in the shoreline survey report available at RIDEM OWR. The geographic limit of the survey was restricted to those sources identifiable from the immediate growing area shoreline within Rhode Island. Thirty-one (31) actual and potential pollution sources were identified within the Kickemuit River. Of these 31 sources, none exhibited elevated fecal coliform counts that required follow-up sampling.

Thirty-nine (39) actual and potential pollution sources were identified in Mt. Hope Bay. Of these 39 sources, two warranted follow-up sampling due to results exceeding the 240 MPN threshold. Subsequent sampling in 2003 indicated that these sources did not impact the receiving waters.

Elevated geometric mean and 90th percentile statistics show an unknown source impacting GA17-3. Potential sources to this station, located near Spar Island, include marine birds and mammals. Evidence suggests that the island is a refuge for waterfowl as well as a seal haul out site. Given the absence of any point sources or other obvious nonpoint sources it is likely that the large concentrations of marine birds and mammals contribute to the excessive fecal coliform values measured at this site.

The City of Fall River, MA has a sewer system with over 176 miles of combined sewers and 11 pump stations. Wet weather causes frequent combined sewer overflows (CSOs) at 19 locations throughout the city that discharge into Mt. Hope Bay, the Taunton River, and the Quequechan River. The Fall River WWTF discharges approximately 1.5 billion gallons per year of untreated and/or partially treated sewage to Mt. Hope Bay (Burns 2001).

Three sewage treatment plants have the potential to influence water quality in the Mount Hope Bay and Kickemuit growing areas. These are Taunton WWTF, Somerset WWTF, and the Fall River WWTF. The Fall River WWTP is permitted to discharge to 19 locations through multiple CSOs into Mt. Hope Bay, the Taunton River, and the Quequechan River. The long-term CSO planning process was initiated in 1984 for the City of Fall River in response to a lawsuit by the Conservation Law Foundation. This process culminated in 1992 with a $130 million dollar Three-Phase plan, which included deep rock tunnels for CSO storage and pumpback, along with upgrades to the wastewater treatment facility. The plan is expected to reduce CSO discharges to four untreated events per year. Currently Phase I of the plan has been completed and has eliminated wet weather discharges from two of the largest CSOs discharging directly to the bay (Mount Hope Ave. and Ferry Street CSOs).

The CSO impact area of Mt. Hope Bay is designated Class SB. The Lee River is classified as SA. There are currently no CSO discharges to the Lee River. The Cole River is classified as SA. There are currently no CSO discharges to the Cole River. The Quequechan River discharges to Mt. Hope Bay near the city of Fall River. The City of Fall River is permitted to discharge via eight wet weather CSOs to the Quequechan River.

A number of municipalities within the Taunton River watershed have implemented measures to address sewage discharges and CSO events. The City of Taunton made upgrades to their wastewater treatment plant (WWTP) in 2001 and 2002. As a result, the number of CSO events has dropped from 24 events in 2000 to only one event in 2004. The City of Fall River has been addressing CSOs since 1984. The city's three phase CSO program includes upgrades to the WWTP, a CSO tunnel to enlarge the storage capacity of the system, and partial sewer and catchbasin separation. The WWTP upgrades and Phase I of the project have been completed. One result of these improvements is that dry weather CSO flows from the city have been virtually eliminated by the spring of 1990 (ASA 1990). Two of the largest CSOs that had previously discharged directly to the bay, south of the city of Fall River, have been eliminated. The City of Brockton received funding from the State Revolving Fund program to reduce sewer system overflows and discharge violations. In 2004, the WWTP began a three phase facility-wide upgrade to improve effluent quality. The Town of Dighton has received funds from the Clean Water SRF to identify areas where the existing onsite sewage disposal systems are inadequate and to develop wastewater management recommendations.

5.5 Proposed Monitoring Strategy

TMDL development will rely, in part, on an adequate dry and wet weather fecal coliform dataset within the study area. Sufficient dry weather data exist; therefore no additional dry weather surveys are necessary. A majority of the wet weather data available within the study area was collected within the 1984-1996 time period. Three wet weather surveys focusing on the Massachusetts portion of the Mount Hope Bay were carried out by ASA in 1990. To date, no other comprehensive wet weather surveys have been conducted in the bay or within the Kickemuit River. The proposed monitoring strategy focuses on assessing the bacteriological condition of the estuarine waters during wet weather events. A subset of sources, prioritized from existing shoreline surveys, will also be sampled. The TMDL will be concentration-based, therefore flow data from sources will not be collected.

The TMDL study will require field data collection and a closer analysis of historical data. Water quality sampling during critical wet weather periods will examine fecal coliform bacteria concentrations in Mt. Hope Bay and the Kickemuit River. DEM Office of Water Resources will coordinate with the Massachusetts Division of Marine Fisheries (MADMF) during wet weather sampling efforts. MADMF will sample shellfish stations within MA waters (MHB1- MHB4), and as shown in Appendices A.6-A.9. Split samples will be collected by MADMF staff at five stations in MHB1 (Stations 1,2,4,5, and 11), picked up by DEM staff, and then analyzed at RIDHL. Results from these samples will be compared to those obtained from the MADMF Laboratory in Pocasset, MA. The general approach regarding the proposed monitoring strategy is provided below.

6.0 Project Description and Schedule

The requirements of the TMDL process help determine the scope of the Mt. Hope Bay and Kickemuit River wet weather studies. The goal of the sampling is to 1) characterize water quality in Mount Hope Bay and the Kickemuit River during wet weather conditions, 2) provide a more robust and up-to-date wet weather fecal coliform dataset that can be combined with existing dry weather data to develop a TMDL for waters within the study area, and 3) sample a subset of pollution sources that were determined to have the greatest potential to impact receiving water quality in the growing areas.

DEM will collect samples from a subset of actual and potential sources for analysis for fecal coliform bacteria and male-specific bacteriophage. Beginning in the spring of 2006, RIDEM will conduct two wet weather surveys during two separate storm events. MADMF Shellfish Sanitation and Management staff will concurrently collect samples from all stations within growing areas MHB1-4. Data collected by the MADMF Shellfish Program will be kept separate from RIDEM data but can be accessed by contacting Susan Boehler, Lab Supervisor at 508.563.1779 xt. 135.

6.1 Tasks

The following tasks outline the steps needed to accomplish the objectives of the sampling program. The tasks relate to wet weather monitoring surveys.

Task 1 Selection of Storm Criteria

Establishing rainfall criteria is critical to the success of the monitoring program and the interpretation of the data. The basic objective is to isolate the effect of a discrete event to permit the characterization of runoff and the determination of the impact on receiving water quality. The following rainfall criteria are proposed for this field program:

- Minimum rainfall of 1.5 inches (3.8 cm) in a 24-hr period
- Minimum duration of 5 hours
- Minimum antecedent dry period (ADP) of 7 days
- Storm should cover a significant portion of the watershed and be based on model predictions.

The minimum rainfall amount of 1.27 cm is an assurance that there will be sufficient rainfall to cause a runoff event and trigger CSO discharges from the city of Fall River. The minimum duration of five (5) hours rules out short, high-rainfall storms, like summer thunderstorms, and directs the storm collection to a more extensive system, which is somewhat easier to forecast and increases the probability of capturing an extensive storm. It is necessary to have pre-storm conditions (baseline) out of the influence of a previous storm or to be essentially steady-state. The selection of the three (3)-day ADP assures for this although it is somewhat arbitrary. The combined watershed area of the Kickemuit River and Taunton River/Mt. Hope Bay is approximately 1475 km^2, therefore the selected storm event should, at the very least, cover a fair portion of the lower watershed.

The Project Manager will keep track of atmospheric conditions and the development of potential storms and inform all samplers when conditions are favorable for a significant precipitation event to occur. Numerous numerical weather prediction models exist that display output on the Internet and forecast events 3-5 days in advance. The Project Manager is familiar with interpreting model output and will access them via the Internet to help target potential wet weather events. The following links provide access to model output and discussion:

http://www.erh.noaa.gov/box/maps.shtml
http://asp1.sbs.ohio-state.edu/
http://weather.unisys.com/ngm/
http://www.erh.noaa.gov/er/box/gfe/gridded.html

Task 2 Pollution Source Monitoring

Samples will be collected from one (1) tributary (the outlet of the freshwater portion of the Kickemuit River), Thirteen (13) point sources to the Kickemuit River and Mt. Hope Bay, and 40 estuarine stations within Mt. Hope Bay and the Kickemuit River. Tributary and outfall locations were selected to provide information about several of the largest potential non-permitted sources of pollution.

The RIDEM OWR Shellfish program conducted sanitary surveys of Mt. Hope Bay and the Kickemuit River in 2002. The geographic limit of the survey was restricted to those sources identifiable from the immediate growing area shoreline within Rhode Island. Thirty-one (31) actual and potential pollution sources were identified within the Kickemuit River and thirty-nine (39) actual and potential pollution sources were identified in Mt. Hope Bay.

A subset (n=13) of tributary and outfall sources to be sampled during this study were selected from the 2002 sanitary surveys of both Mt. Hope Bay and the Kickemuit River. Sources were chosen based on flow, size of outfall, fecal coliform concentration, and existing land use. The intent of sampling a subset of sources is to identify the major sources of fecal coliform and to assess the bacterial loading associated with different land uses.

All source stations will be sampled at two (2), six (6), and twelve (12) hours after the beginning of the storm. Sources will only be sampled when they are flowing. Samples will be analyzed for fecal coliform, using the MPN method, and male-specific bacteriophage using RIDHL methodology. The latter analysis serves as a screening tool to evaluate the presence of human sewage. The tributary station, located at the mouth of the freshwater portion of the Kickemuit River has a longer travel time and will be sampled before the storm and every twelve hours after the start of the precipitation event until hour 60.

Table 6.1 provides information about sampling and analysis methods. Appendix A.1 provides additional detail regarding the sampling location and monitoring protocol for each station and Appendix A.2 shows the geographic locations of sources. Appendix A.3 provides more detailed information on sampling locations and directions to sites.

Table 6.1 Analytical Services Table for Mt. Hope Bay and Kickemuit River Sources.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	Number of Sampling Locations[1]	Number of Field Duplicates	Total number of Samples to Lab.	Data Package Turnaround	Laboratory Name
Surface/ Stormdrain	Fecal Coliform	MPN	78	4	82	10 Days	RIDHL
Surface/ Stormdrain	Male-specific bacteriophage	Att. B	78	4	82	10 Days	RIDHL

[1]Samples taken at separate times at the same location count as a separate sampling location/station. Total number of samples is the number for both wet weather surveys. (13 stations X 3 samples per station = 39 X 2 storm events = 78) + 5% duplicates = 82.

Task 3 Mt. Hope Bay and Kickemuit River estuarine monitoring

With the exception of the single sampling station added for purposes of this wet weather study, all proposed sampling stations within GA5 and GA17 are current FDA-approved Shellfish sampling stations. These stations were selected by the RI Shellfish Program to represent bacteriological conditions within

each growing area. The US Food and Drug Administration (FDA) who administers each state's program approves the geographic location of all shellfish sampling stations. One new sampling station was added to evaluate the near-shore area off Roger Williams University in Bristol. All estuarine stations will be sampled by boat. Analysis for samples will be by the MPN method at the RIDHL. Sampling will occur for four of the next five days after the end of the storm. Appendix A.4 details the station descriptions. The locations of the stations are also shown in Appendices A.5 and A.6. Data collected by the MADMF Shellfish Program will be kept separate from RIDEM data but can be accessed by contacting Susan Boehler, Lab Supervisor at 508.563.1779 xt. 135.

MADMF staff will be sampling stations in growing areas MHB1-MHB4 concurrently during each precipitation event. Analysis for samples collected in MA waters will be by the MPN method at the MADMF Lab in Pocasset, MA. RIDEM will split samples with MADMF staff at five stations located within MA growing area MHB1 during each study for analysis at RIDHL. Table 6.2 contains information about sampling and analysis methods.

Table 6.2 Analytical Services Table for Mt. Hope Bay and Kickemuit River Estuarine Stations.

Medium/ Matrix	Analytical Parameter	Analytical Method/ SOP	No. of Sampling Locations[1]	No. of Field Duplicates	Total No. of Samples to Lab	Data Package Turnaround	Laboratory Name
Surface water	Fecal Coliform	MPN	384	20	404	10 Days	RIDHL

[1]Refers to total number of samples from all stations in GA17 and 5 and includes one (1) new station, as well as five (5) stations in MA MHB1 growing area.

6.2 Project Schedule

Table 6.3 Project Schedule.

Task	Deliverable	2006					
		M	J	Jul	A	S	O
Site Preparation	NA						
Sample Collection	Spring-Fall 2006						
Laboratory Analysis	Spring-Fall 2006						
Final Data Report	Fall 2006						

7.0 Project Quality Objectives and Measurement Performance Criteria

Collecting high quality data is one of the most important goals of this project. Specific data quality objectives include method detection limits, precision, accuracy, representativeness, comparability, and completeness. All the data quality objectives will be met if the data collected are sufficient to complete the TMDL.

7.1 Measurement Performance Criteria

Representativeness

As stated previously, the selected stations were chosen for their representativeness of conditions in the study area. The sampling frequency was chosen to characterize bacteriological conditions during and immediately following the storm to evaluate impacts from both near shore and more distant watershed sources of bacteria. The sampling targets wet weather to address identified data gaps and because historically, this is when fecal coliform bacteria levels were observed to exceed state water quality criteria. The extent to which the measurements represent actual environmental conditions will be somewhat restricted by the time of year the samples are taken and the overall weather conditions of that year (i.e. wet versus dry year).

Comparability

To maximize the quality of the data collected, and to collect data that is comparable with other studies, accepted sampling procedures will be used during this study. All samples collected will be sent to FDA-Approved Laboratories (RIDOH Lab in Providence, or MADMF Lab in Pocasset, MA). This study uses one method to analyze samples for fecal coliform bacteria. The MPN method will be used to analyze samples taken from Mt. Hope Bay and the Kickemuit River, as well as source samples.

Sensitivity

Analytical methods were selected such that detection limits will not limit the usefulness of the data set.

Completeness

If the data collected is sufficient to complete the TMDL report, then the data is considered to be complete. Measurement performance criteria help determine the completeness of a data set. Tables 7.1 and 7.2 document the measurement performance criteria for this project.

Table 7.1 Measurement Performance Criteria- Fecal Coliform.

Sampling SOP	S-1
Medium/Matrix	Surface Water
Analytical Parameter	Fecal Coliform
Concentration Level	<1

Data Quality Indicator	Analytical Method/ SOP Reference/ Laboratory	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Measurement Performance	QC Sample Assesses Error for Sampling (S), Analytical (A), or both (S/A)
Precision	MPN/ Standard Method 9221BD/ RIDHL	Within 95% Confidence Interval	Field Duplicates/Split	S/A
Accuracy/bias Contamination	MPN/ Standard Method 9221BD/ RIDHL	Positive Growth (>2)	Method Blank	A
Accuracy/bias Contamination	MPN/ Standard Method 9221BD/ RIDHL	No Growth	Reagent Blank	A
Data - Completeness	MPN/ Standard Method 9221BD/ RIDHL		Anticipate 100%	A
Accuracy	MPN/ Standard Method 9221BD/ RIDHL	Within 95% Confidence Interval	Field Duplicates/Split	S/A

Table 7.2 Measurement Performance Criteria- Male Specific Bacteriophage.

Sampling SOP	S-1
Medium/Matrix	Surface Water
Analytical Parameter	Male-Specific Bacteriophage
Concentration Level	< 1 / 100 ml

Data Quality Indicator	Analytical Method/ SOP Reference/ Laboratory	Measurement Performance Criteria	QC Sample and/or Activity Used to Assess Measurement Performance	QC Sample Assesses Error for Sampling (S), Analytical (A), or both (S/A)
Precision	Dbl. Agar Overlay/ NELEOM/ FDA		Field Duplicates/Split	S/A
Accuracy/bias Contamination	Dbl. Agar Overlay/ NELEOM/ FDA	Plaque Formation	Positive Control	A
Accuracy/bias Contamination	Dbl. Agar Overlay/ NELEOM/ FDA	No Plaque Formation	Reagent Blank	A
Data - Completeness	Dbl. Agar Overlay/ NELEOM/ FDA		Anticipate 100%	A
Accuracy	Dbl. Agar Overlay/ NELEOM/ FDA	Plaque Formation	Duplication of Spike control	S/A

8.0 Sampling Process Design

8.1 Sampling Design Rationale

Task 1 Pollution Source Sampling

Appendices A.1-A.3 describe the exact location and monitoring protocol for all source stations. In general, sampling of selected sources will occur at 2, 6, and 12 hrs following the start of the event. To ensure that the freshwater Kickemuit River station returns to pre-storm levels, sampling will extend for up to four days (pre-storm, 12, 24, 48, 72, and 96 hrs). RIDHL will use the MPN analysis method to analyze all source samples for fecal coliform bacteria (Attachment B.1) and the SOPs provided in Attachment B.2 for male-specific bacteriophage analysis. Table 8.1 contains information about sampling and analysis methods.

Table 8.1 Source Sampling and Analysis Method/SOP Requirements.

Lab	Medium/ Matrix	Depth	Analytic Parameter	SOP		Container[2]			Container[2] Req.	Temp	Holding Time
				Sampling	Analytical	No.	Size	Type			
RIDHL	Water	2-12 inches	Fecal Coliform	S-1	MPN	1	125 mL	Polyethylene	Ice	4°C	8 Hours
RIDHL	Water	2-12 inches	Male-specific bacteriophage	Att. B	Att. B	1	125 mL	Polyethylene	Ice	4°C	8 Hours

[2]The laboratory that completes the sample analysis will provide sterile bottles.
[3]Some source samples (collected to determine relative magnitude of sources and not for compliance purposes) may be held for up to 24-hrs until analysis depending on the timing of the storm relative to the laboratory's hours of operation. Those samples with a longer than 6-hr holding time will be specifically identified in the final data report.

Task 2 Estuarine Sampling

All estuarine sampling stations are the same as RI Shellfish Program stations, with the exception of the one station added for this survey. Appendices A.5 and A.6 describe the exact location and monitoring protocol for each estuarine station, including the two added stations. All estuarine stations will be sampled approximately 12, 24, 48, 72, and 120 hours after the beginning of the precipitation event.

The RIDHL will use the MPN method to analyze all marine samples. The Food and Drug Administration requires that any samples, which may be used to reopen a Shellfish Area be analyzed using the MPN Method at a certified laboratory. The RIDHL is the only certified laboratory in Rhode Island. Table 8.2 contains information about sampling and analysis methods. Split samples will be collected by MADEP staff within GA MHB1 with analyses being performed by RIDHL and MA Shellfish Lab in Pocasset, MA. Table 8.2 contains information about sampling and analysis methods.

Table 8.2 Estuarine Sampling and Analysis Method/SOP Requirements.

Lab	Medium/ Matrix	Depth	Analytic Parameter	SOP		Container[2]			Container[2] Req.	Temp	Holding Time
				Sampling	Analytical	No.	Size	Type			
RIDHL	Surface Water	6-12 inches	Fecal Coliform	S-1	MPN	1	125 mL	Polyethylene	Ice	4°C	8 Hours

[2]The laboratory that completes the sample analysis will provide sterile bottles.

9.0 Sampling Procedures and Requirements

9.1 Sampling Procedures

Standard operating procedures for field sampling are located in Attachment A.1 of this report.

Table 9.1 Project Sampling SOP Reference Table.

Reference Number /Title	Originating Organization	Equipment Identification	Modified for Work Project	Comments
Field Sampling SOP 1 (S-1) Fecal Coliform and Male-Specific Bacteriophage Sampling	RIDEM	Not Applicable	No	

9.2 Equipment Cleaning

RIDHL will provide sterile bottles for collecting samples for fecal coliform and male-specific bacteriophage analysis.

9.3 Field Equipment Maintenance

The Project Manager will ensure that all field equipment is operating properly. The only equipment needed for sampling is a 23-foot Ocean Scout, typically maintained and operated by RIDEM Shellfish Unit staff. Other equipment would typically include associated safety and maintenance gear, and sampling equipment.

10.0 Sample Handling, Tracking, and Custody Requirements

10.1 Field Notes and Sample Tracking

Water Quality Monitoring

Two sampling teams, consisting of two persons per team, will be needed for each wet weather survey. An additional person will be necessary to transport samples from the field to the DOH Laboratory within the 8 hour holding time. Some source samples (collected to determine relative magnitude of sources and not for compliance purposes) may be held for up to 24-hrs until analysis depending on the timing of the storm relative to the laboratory's hours of operation. Those samples with a longer than 8-hr holding time will be specifically identified in the final data report. Every effort will be made to meet the 8-hr holding time, however under a "worst case scenario" and consistent with Standard Methods 9060 B. Instructions for each team are provided below.

Field Notes and Sample Tracking

All sampling teams will be provided with a field notebook. Each team member should ensure that a log of events is faithfully and articulately maintained in one of the notebooks used to document field studies. A minimum log includes the date, samplers name, station location, sample name and run (wet weather), sample collection times, and any other significant information (tide, wind direction and speed, any source observations, etc.).

The proper identification of the sample is important. Before it is filled, the sample bottle should be labeled with the following information: sample station, date of collection, time of collection (e.g. 0700hrs, 1300hrs, etc.). Additionally, a number corresponding to the sample run should be inserted. For example, a sampler collecting the pre-storm sample at 0700 hrs at station GA17-3 would label the bottle "GA17-3-0700P. A sampler collecting a 48 hr sample at 1300 hrs at station GA17-9 would label the bottle "GA17-9-1300-48". Any replicate samples would have an "R" after the label. When taking the sample, the sampler should fill in the Sample ID on both the notebook and the sample bottle label. Figure 10.3 depicts the Sample Bottle Label.

The 'SAMPLE #, DATE/TIME, COLLECTION POINT, and COLLECTOR' fields should be filled in prior to sampling. Each sample bottle will also be given a laboratory number. RIDEM personnel will write this number on both the Sample Bottle and the chain of custody form. The bottle should be labeled with permanent marker prior to taking the sample. It is difficult to write on wet sample bottles. Note that no chlorinated samples will be collected. Therefore, the THIO or thiosulfate field on the sample bottle is not needed. At the completion of sampling, all log sheets and notebooks will be turned over to the Project Manager.

RI Department of Health Laboratory ( ) **Legal**
Sample #_____ **Date/Time**_____
Collection point_____
Collector_____ **Program**_____
Preservatives added:_____ **THIO** **Sterilized on:**
(X) By Lab

Figure 10.3 RIDHL Sample Bottle Label

Appendix B.1 presents the chain of custody form for the RIDHL. Before the samples are handed over to the laboratory, all fields must be filled in, especially the sample ID and the Laboratory Number. The laboratory and RIDEM are given a copy of the completed chain of custody form.

Sample Handling

All samples will be placed in a cooler with ice immediately after the sample is taken. The sample will be delivered to the laboratory within six hours. As long as some ice is still present in the cooler when it arrives at the lab, it will be assumed that the sample arrived to the lab at the correct temperature (4 ^{o}C). A designee of the Project Manger will deliver the samples to the laboratory. Table 10.1 documents the sample handling system.

Table 10.1 Sample Handling System.

	Responsible Party	Samples
Sample Collection	RIDEM and designees	Source and Marine/Estuarine
Sample Delivery	RIDEM	Source and Marine/Estuarine
Sample Analysis	RIDOH Laboratory	Source
	RIDOH Laboratory	Marine/Estuarine
Sample Archival	None	Not Applicable
Sample Disposal	RIDOH Laboratory	Source and Marine/Estuarine

11.0 Field Analytical Method Requirements

During sampling, no field analyses will take place.

12.0 Fixed Laboratory Analytical Method Requirements

All samples taken from both marine and source waters will be taken to RIDHL in Providence, Rhode Island. Estuarine samples will be analyzed for fecal coliform bacteria using the MPN method. Source samples will be analyzed for both fecal coliform bacteria using the MPN method and for male-specific bacteriophage using standard operating procedures detailed in Attachments B.1 and B.2. Table 12.1 provides the fixed laboratory analytical method/SOP reference table for RIDHL.

Table 12.1 Fixed Laboratory Analytical Method/SOP Reference Table.

Reference Number	Fixed Laboratory Performing Analysis	Title	Definitive or Screening Data	Analytical Parameter	Instrument	Modified for Work Project
L-1	RIDHL	MPN Method for Detection of Fecal Coliform.	Definitive	Fecal Coliform	NA	N
L-2	RIDHL	Procedure for Determining Male-specific Bacteriophage In Seawater, Wastewater and Shellfish Samples	Definitive	Male-specific bacteriophage	NA	N

13.0 Quality Control Requirements

Table 13.1 Field Sampling QC: Fecal Coliform.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Fecal Coliform/Male-specific bacteriophage
Concentration Level	<1
Analytical Method/ SOP Reference	S-1

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Field Duplicates	Minimum 1 per 20 samples	L-1	Discuss any problems in the field with sampler.	Project Manager	Precision	Within 95% Confidence Interval

Table 13.2 Fixed Laboratory Analytical QC: Fecal Coliform Bacteria. RI Dept. of Health Laboratory.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Fecal Coliform
Concentration Level	<1
Analytical Method/ SOP Reference	Standard Method 9213D

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Method Blank	1 Per Batch	L-1	Reprepare Batch	Kerry Patterson	Bias- Contamination	Positive Growth (>2)
Reagent Blank	1 Per Batch	L-1	Reprepare Batch	Kerry Patterson	Bias- Contamination	No Growth
Laboratory Duplicate	1 per 10 samples	L-1	Reanalyze	Kerry Patterson	Precision-Lab	Within 95% Confidence Interval

Table 13.3 Field Sampling QC: Male-Specific Bacteriophage.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Male-specific bacteriophage
Concentration Level	<1
Analytical Method/ SOP Reference	See Attachment B

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Field Duplicates	Minimum 1 per 20 samples	L-2	Discuss any problems in the field with sampler.	Project Manager	Precision	Within 95% Confidence Interval

Table 13.4 Fixed Laboratory Analytical QC: Male-Specific Bacteriophage. RI Dept. of Health Laboratory.

Sampling SOP	S-1
Medium/ Matrix	Surface Water
Analytical Parameter	Male-specific bacteriophage
Concentration Level	<1
Analytical Method/ SOP Reference	See Attachment B

QC	Frequency/ Number	Method/SOP QC Acceptance Limits	Corrective Action	Person Responsible for Corrective Action	Data Quality Indicator	Measurement Performance Criteria
Method Blank	1 Per Batch	L-2	Reprepare Batch	Kerry Patterson	Bias- Contamination	Positive Growth (>2)
Reagent Blank	1 Per Batch	L-2	Reprepare Batch	Kerry Patterson	Bias- Contamination	No Growth
Laboratory Duplicate	1 per 10 samples	L-2	Reanalyze	Kerry Patterson	Precision-Lab	Within 95% Confidence Interval

14.0 Data Acquisition Requirements

The existing fecal coliform dataset consists of over twenty years of monthly grab samples collected by RIDEM OWR Shellfish Program staff at 26 stations within the two growing areas. From 1984 to 1996 samples were collected during both dry and wet weather. Following the reclassification of these two growing areas in 1996 to "Conditionally Approved", only dry weather data were collected. The last comprehensive wet weather study within Mt. Hope Bay was conducted by Applied Science Associates (ASA) Inc. in 1990, as part of a larger study designed to assess the City of Fall River's Combined Sewer Overflow Facilities Plan

The RIDEM OWR Shellfish program conducted a sanitary survey of Mt. Hope Bay and the Kickemuit River in 2002. The geographic limit of the survey was restricted to those sources identifiable from the immediate growing area shoreline within Rhode Island. Thirty-one (31) actual and potential pollution sources were identified within the Kickemuit River. Of these 31 sources, none exhibited elevated fecal coliform counts. Thirty-nine (39) actual and potential pollution sources were identified in Mt. Hope Bay. Of these 39 sources, two warranted follow-up sampling due to results exceeding the 240 MPN threshold. Subsequent sampling in 2003 indicated that these sources did not impact the receiving waters.

A major limitation to the above-mentioned data collection and source analysis investigations is that they were not conducted during wet weather conditions. Aside from the 15-year old investigation by ASA Inc. conducted in 1990, no comprehensive wet weather dataset exists.

RIDEM will use rainfall information from the National Weather Service stations in Taunton, Massachusetts, as well as T.F. Green Airport in Warwick, RI. The Taunton station is located near the Watson Pond State Park, within the Taunton River/Mt. Hope Bay watershed and approximately 22km north of Mount Hope Bay proper. The T.F. Green station is located in Warwick, approximately 20 km due west of Mount Hope Bay proper. Table 14.1 summarizes non-direct measurements used in setting up the Mt. Hope Bay and Kickemuit River wet weather study.

Table 14.1 Non-Direct Measurements Criteria and Limitations.

Non-Direct Measurement (Secondary Data)	Data Source	Data Generator	How Data Will Be Used	Limitations on Data Use
Rainfall	http://www.erh.noaa.gov/box/dailystns.shtml	National Weather Service	Quantify amount of rainfall received in watershed.	None. Use judiciously
Sanitary Survey of Sources	Mt. Hope Bay and Kickemuit River, RI Shellfish Growing Area Survey and Classification Considerations	RIDEM	Rank fecal coliform sources. Evaluate impact on estuarine water quality.	Data collected in 2002 No comprehensive monitoring of sources.
Bacteriological Monitoring	ASA Inc. wet weather survey of Mt. Hope Bay	Applied Science Associates Inc.	Initial wet weather assessment	Data is 15 years old.
Bacteriological Monitoring	Shellfish Surface Water Monitoring Program	RIDEM Shellfish Surface Water Monitoring Program	Routinely evaluate bacteriological condition of growing area waters.	Dry weather (< 0.5" of rainfall) sampling only.

15.0 Documentation, Records, and Data Management

All samplers will be given a field notebook. The monitoring plan that will be distributed when each sampler collects his/her equipment includes specific information on what needs to be recorded in the notebook. All log sheets will be given to field leader at the conclusion of sampling. Initials on these sheets identify the sampler. The Project Manager will review the sheets within three days to identify any possible errors or omissions. The Project Manager will contact any sampler whose sheet shows any discrepancies. In addition, the Project Manager will try to contact all samplers to identify any problems or additional feedback that would make future sampling easier.

The Project Manager will designate a person to collect samples from the samplers during the storm. Each sampler will be responsible for filling out the chain of custody sheets (Appendix B). When the samples are picked up from the samplers, the Project Manager or designee will check the chain of custody sheets. The samples and chain of custody sheets are also checked at the laboratory. A copy of the chain of custody form will be given to RIDEM when the samples are dropped off at the laboratory. After analysis is complete, sample results from the laboratory will be mailed to RIDEM.

After each sampling report, a brief Status Report will be written to document any changes to the Monitoring Plan. All information collected throughout the project will be summarized in the Final Data Report. Information included in the Final Data Report is described in Section 17.0. Table 15.1 lists records that will be generated throughout this project. The Project Manager is responsible for the storage of all project files. RIDEM has a central filing system at its Providence Office where all original documents will be kept.

Table 15.1 Project Documentation and Records.

Sample Collection Records	Field Analysis Records	Fixed Laboratory Records	Data Assessment Records
Field Notes/Log Sheets	Field Notes/Log Sheets	Chain of Custody Records	Status Reports
Chain of Custody Records		Tabulated Data Summary Forms: draft and final	Final Data Report
Monitoring Plan			

16.0 Assessments and Response Actions

The Project Manager or designee will be responsible for each of the project tasks and their associated quality assurance and quality control procedures. The Project Manger will provide consistency between sampling events and sampling teams. Continual reports to the QA Officer about the status of sampling, quality assurance, and quality control will highlight any problems that are encountered during sampling. If needed, the QA Officer and Project Manager will halt sampling until problems are remedied.

Table 16.1 Project Assessment Table.

Assessment Type	Frequency	Internal or External	Person Responsible for Performing Assessment and Implementing Corrective Actions	Person Responsible for Monitoring the Effectiveness of the Corrective Action
Field Sampling Technical Systems Audit	Start of Sampling	I	Scott Ribas RIDEM	Brian Zalewsky RIDEM
RIDH Technical Systems Audit	Prior to Sample Receipt	E	Kerry Patterson RIDH Laboratory	Scott Ribas RIDEM

17.0 QA Management Reports

Table 17.1 lists the QA Management Reports that will be generated throughout this study.

As needed during this project, the Project Manager and the QA Officer will meet to discuss any issues related to sampling. These meetings will be verbal status reports. Problems encountered in the field will be discussed and any appropriate actions determined and implemented. Any changes and/or problems will be included in the final report.

After each wet weather event, the Project Manager will generate a Status Report. This Status Report will be the written record of any changes to the QA Plan. If a station was not sampled, it will be documented here. Issues discussed during the Verbal Status Report can also be included.

At the completion of all three events, the Project Manager will write a final report summarizing the three sampling events. Information in this final report will include the following information:

- Brief description of each sampling event
- Data tables of all data collected during the sampling event (including rainfall)
- Attachments
  - Status Reports
  - Sampling Logs
  - Chain of Custody forms
  - Laboratory data sheets provided by the labs

Table 17.1 QA Management Reports.

Type of Report	Frequency	Person(s) Responsible for Report Preparation	Report Recipient
Verbal Status Report	As needed	Scott Ribas RIDEM	Brian Zalewsky RIDEM
Written Status Report	After each wet weather survey	Scott Ribas RIDEM	Brian Zalewsky RIDEM
Final Report	Completion of sampling	Scott Ribas RIDEM	Brian Zalewsky RIDEM

18.0 Verification and Validation Requirements

Both the Project Manager and the QA Officer will review all data collected during this study to determine if the data meets QAPP Objectives. Decisions to qualify or reject data will be made by the Project Manager and QA Officer based on the criteria included in Tables 7.1 and 7.2. All data collected will be included in the Final Report. To ensure correct interpretation of the data, all problems encountered in the field will be included in an Appendix to the report and discussed in the general text of the report. Problems will also be documented in each survey's written Status Report. To assist in data interpretation, statistical information on sampling events, including sampling size, sample mean, and sample variance, will be reported, where applicable. A discussion on duplicate precision and accuracy criteria and results will also be discussed in the Final Report.

19.0 Verification and Validation Procedures

All data collected during the wet weather studies will be included in the appendix of the report. Once the data has been collected, it will be entered into Microsoft Excel files. The Project manager will proofread the data entry for errors. Errors will be corrected. Outliers and inconsistencies will be flagged for further review with the QA Officer. The decision to discard data will be made by the Project manager and QA Officer. Problems will be discussed in the Final Report. Table 19.1 discusses the data verification process.

Table 19.1 Data Verification Process.

Verification Task	Description	I/E	Responsible for Verification
Field Notes	Field notes will be collected at the end of each day and reviewed. Any required corrective actions will be addressed with the field samplers prior to further sampling. After the field notes have been entered into Excel, the data will be proofread for any data entry errors. Copies of the field notes will be maintained in the project file.	I	Scott Ribas/RIDEM
Chain of Custody Forms	Chain of custody forms will be reviewed when samples are collected for delivery to the laboratory in the field and at the laboratory. The forms will be maintained in the project file.	I/E	Scott Ribas/RIDEM Kerry Patterson/RIDHL
Laboratory Data	All laboratory data packages will be verified internally by the laboratory performing the work for completeness prior to submittal. The data packages will also be reviewed by the sampling organization.	I/E	Scott Ribas/RIDEM Kerry Patterson/RIDHL

20.0 Data Usability/Reconciliation with Project Quality Objectives

As soon as possible after each sampling event, calculations and determinations for precision, completeness, and accuracy will be made and corrective action implemented if needed. If data quality indicators meet those measurement performance criteria documented throughout this QA Plan, the project will be considered a success. If there are data that do not meet the measurement performance criteria established in this QA Plan, the data may be discarded and sampled again or the data may be used with stipulations written about its accuracy in the Final Report. The cause of the error will be evaluated. If the cause is equipment failure, calibration/maintenance techniques will be reassessed and improved. If the problem is sampling team error, retraining will occur. Any limitations with the data will be documented in the Status Reports and the Final Report.

References

ASA (Applied Science Associates). 1990. Combined Sewer Overflow Facilities Plan: Receiving Water Impacts Field Measurement Program. ASA Publication #90-20.

Burns, D. 2001. *Fall River CSO, Narragansett /Mt. Hope Bay Watershed.* Southeast Regional Office. Lakeville, MA.

Rippey, Scott R. and Watkins, William D. 1988. Mt. Hope Bay Microbiological Sanitary Survey. U.S. Public Health Service Food and Drug Administration. Northeast Technical Services Unit. Davisville, RI 02852.

Appendix A Sampling Station Information

Appendix A.1. Monitoring Protocol and Sampling Rationale for Pollution Source Stations.

RIDEM TMDL Source ID	Latitude	Longitude	General Location	Drainage Area Characteristics	Stream Or Outfall	Time of Sample Collection (hours) 0 2 6 12	Sampling Rationale
MOUNT HOPE BAY							
MHBS1	41° 38.721'	71° 15.518'	Bristol; Roger Williams College campus	Wetland, R.W. college campus	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
MHBS2	41° 39.890'	71° 15.166'	Bristol; Bristol Landing, nr N. end of Sequoia Dr.	Wetland, condominiums	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
MHBS3	41° 41.588'	71° 14.730'	Bristol; Terminus of Sunrise Dr.	High-density residential	36-inch outfall	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
MHBS4	41° 41.95'	71° 14.36'	Warren; Maple Av.	Ag , wetland, low-density residential	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
MHBS5	41° 39.823'	71° 12.067'	Tiverton; near terminus of Summerfield La.	High-density residential	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
MHBS6	41° 38.911'	71° 12.448'	Tiverton; the Villages at Mount Hope Bay	Large condominium development	10' X 4' outfall	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS1	41° 43.304'	71° 15.807'	Warren; north side of terminus of Parker Av.	High density residential	42" X 20" box culvert	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KICKEMUIT RIVER							
KRS1a	41° 43.304'	71° 15.807'	Warren; south side of terminus of Parker Av.	High density residential	30" culvert	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS2	41° 43.442'	71° 15.872'	Warren; north of terminus of Adams La.	Cattle farm, HD res, wetland, industrial	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS3	41° 43.521'	71° 15.872'	Warren, north of terminus of Libby La.	High density residential	18-inch outfall	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS4	41° 43.561'	71° 15.838'	Warren; S of railroad bridge at terminus of Barker Av.	High density residential	Stream	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS5	41° 43.754'	71° 15.760'	Warren; west of Child St. bridge	High density residential	18-inch outfall	X X X X	Confirm as significant wet weather source and evaluate presence of sewage
KRS6	41° 43.756'	71°15.758'	Warren; west of Child St. bridge	Outflow of the freshwater portion of the Kickemuit River	18-inch outfall	12 24 48 72	Assess input of freshwater portion of the Kickemuit River

Appendix A.2. Map of Pollution Source Stations.

Appendix A.3. Source Sampling Location Descriptions.

MHBS1
This stream is located in Bristol, on the campus of Roger Williams University. This stream discharges to Mt. Hope Bay approximately 1700 ft south-southwest of the Roger Williams University dock.

MHBS2
This stream is located in Bristol east of Bristol Landing condominium development, approximately 1300 ft. south-southeast of the intersection of Metacom Av. (Route 103) and Weetamoe Farm Rd. The stream is best accessed from a grassed cart path near the northern terminus of Sequoia Ct. The stream will be sampled at a stone culvert under the stonewall-lined cart path, approximately 375 ft. east of a stone gate located near the northern end of Sequoia Ct.

MHBS3
This 36-inch culvert is located in Bristol at the terminus of Sunrise Dr.

MHBS4
This stream is located in Warren and discharges to Mt. Hope Bay approximately 500 ft. southeast of the cul-de-sac of Blackthorne La. The stream will be sampled at Maple Rd. in Warren. The stream is culverted along the northern edge of Maple Rd. The up-gradient end of the stream is located approximately 300 ft. east of the intersection of Maple Rd. and Touisset Rd. at Utility Pole No. 7. The down-gradient end of the stream is located approximately 200 ft. east of the intersection of Maple Rd. and Touisset Rd. at Utility Pole No. 4.

MHBS5
This stream discharges to Mt. Hope Bay approximately 300 ft. west of the cul-de-sac of Summerfield La. The stream is best accessed from a right-of-way near then intersection of Colony Ter. and Robert Gray Av. Proceed northerly along abandoned railroad bed for approximately 800 ft. to the stream crossing.

MHBS6
This 10' X 4' box culvert discharges to Mount Hope Bay at the Villages at Mount Hope Bay condominium development. The culvert is associated with a large concrete structure and is located approximately 800 ft north-northeast of a 400 ft dock.

KRS1
This short stream originates from a 42" X 20" box culvert **(KRS1)** and a 30"culvert **(KRS1a)** at the terminus of Parker Av. in Warren.

KRS2
This stream discharges to the western bank of the Kickemuit River approximately 450 ft south-southeast of the cul-de-sac of Libby La. in Warren

KRS3
This 18-inch culvert is located in Warren approximately 250 feet east-northeast of the cul-de-sac of Libby Ln.

KRS4
This stream is located in Warren on the west bank of the Kickemuit River, at the southern side of a broken railroad bridge near the terminus of Barker Av. This short stream originates at a culvert that is inaccessible.

KRS5
This 18-inch culvert is located in Warren approximately 35 ft. southwest of the western wing wall of the Child St. (Route 103) bridge.

KRS6
This 18-inch culvert is located in Warren approximately 20 ft. southwest of the western wing wall of the Child St. (Route 103) bridge. This culvert is the outlet to the freshwater portion of the Kickemuit River.

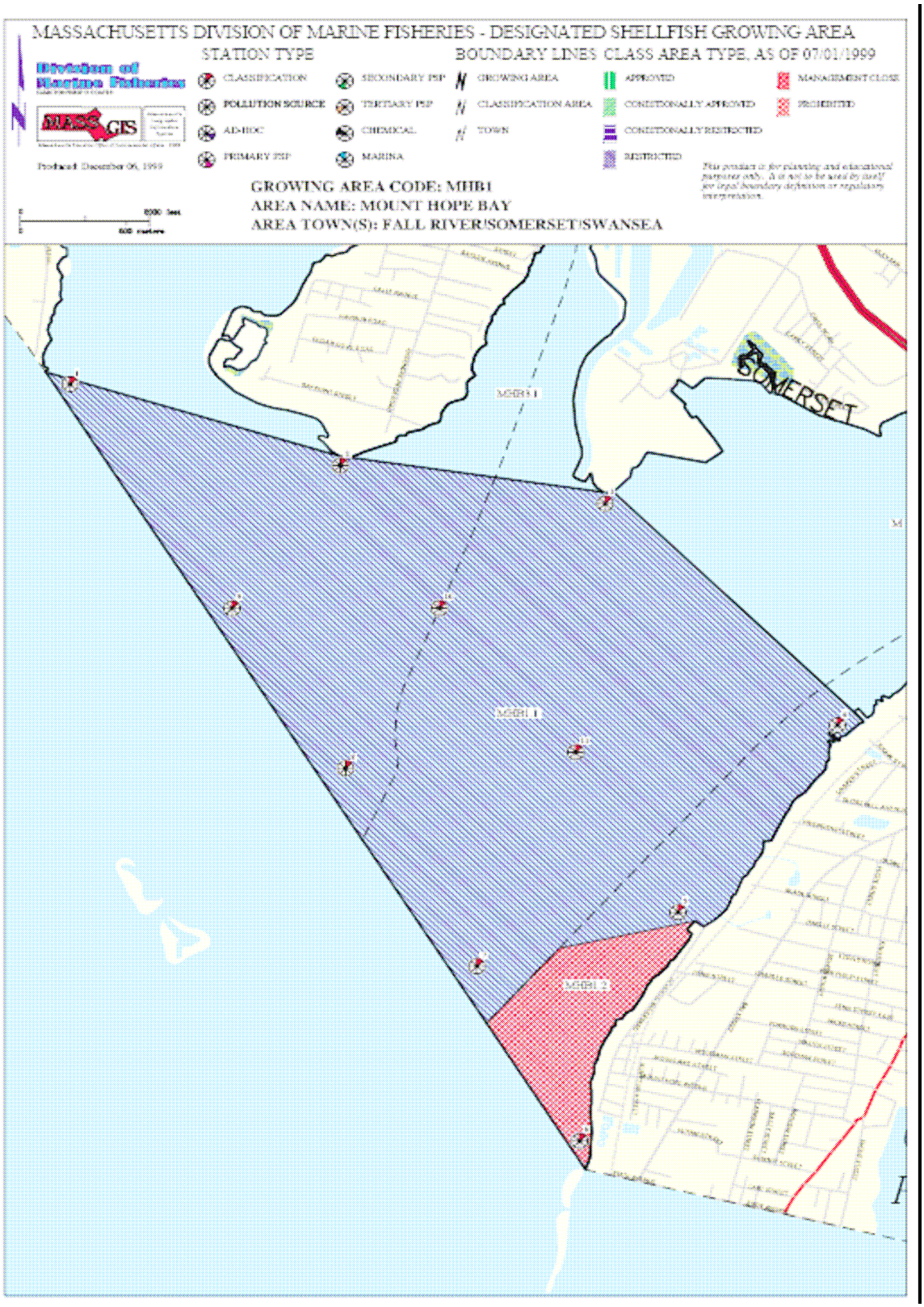
Appendix A.4. Monitoring Protocol and Sampling Rationale for Estuarine Stations.

Station	Location		Monitoring Protocol Time of Sample (hrs)						Sampling Rationale
RI- Mt. Hope Bay			**P**	**12**	**24**	**48**	**72**	**120**	
GA17-1	The intersection of a line from Nun Buoy #2 west of Spar Island through Can Buoy #1 at Old Bay Rock, and the extension of a line from Nun Buoy #4 at Bristol Narrows through the seaward end of the longest dock at Toweset Pt.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-2	The intersection of a line from Can Buoy #1 at Old Bay Rock to the center stack at the Brayton Pt. power plant, and a line from the center of Coles River to microwave tower over N.E. Petroleum tank farm.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-3	At Nun Buoy #2 just west of Spar Island	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-4	At Can Buoy #7	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-5	At red Bell Buoy #4	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-6	At Can Buoy #5	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-7	At Gong Buoy #3	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-8	Just off north dock at N.E. Petroleum Company	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-9	Just north of the center structure of the Tiverton-Portsmouth railroad bridge.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-10	At Can Buoy #19 off Common Fence Point	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-11	The intersection of a line from the point of land on the western side of Common Fence point to the southern abutment of the Mt. Hope Bridge, and the extension of a line from the eastern shore of Mt. Hope Pt. through Nun Buoy #2.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-12	On northeast side of southern abutment of the Mt. Hope Bridge	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-13	The intersection of a line from the southern abutment of the Mt. Hope Bridge to the eastern shore of Mt. Hope Point, and a line from the northern abutment of the Mt. Hope Bridge to RG MH Fl Gong Buoy	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-14	The intersection of a line from the southern tip of Mt. Hope Pt. to the southern tip of Bristol Pt. and a line from Seal Island to RG MH Fl Gong Buoy.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-15	At Nun Buoy #2	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-16	Approximately 100 yards east off the earthen dock at the end of Hopeworth Street.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA17-13A	Approximately midway along a straight line drawn between GA17-14 and GA17-13	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
RI- Kickemuit River			**P**	**12**	**24**	**48**	**72**	**120**	
GA5-1	At Nun Buoy #2 at the mouth of the Kickemuit River.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-2	The intersection of a line from Nun Buoy #2 at the mouth of the Kickemuit River and Can Buoy #1 at Old Bay Rock, and a line from Nun Buoy #6 at the mouth of the Kickemuit River to Nun Buoy #2 at Spar Island.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-3	At Nun Buoy #6 at the mouth of the Kickemuit River.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-4	The intersection of a line from Narrows Rock to the smokestack in Warren, and a line from the western end of Smith Street to Little Neck Point.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-5	The intersection of a line from Narrows Rock to the smokestack in Warren, and the extension of Sherman Avenue.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-6	The intersection of a line from Narrows Rock to the smokestack in Warren, and a line from the western end of Harris Avenue to the tallest tower at the Brayton Point Power Plant.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-7	At a point in mid-channel east of the Laurel Park pavilion	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-8	At the most northerly point obtainable in the Kickemuit River.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-9	The intersection of a line from the building at the crest of Poor Farm Hill to Narrows Rock, and a line from the western end of Harris Avenue to the tallest tower at the Brayton Point Power Plant.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
GA5-10	The intersection of a line from the building at the crest of Poor Farm Hill to Narrows Rock, and the westerly extension of the most southerly dock in Touisset Highlands.	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
MA- Mt. Hope Bay	**SPLIT SAMPLE LOCATIONS**		**P**	**12**	**24**	**48**	**72**	**96**	
MHB1		In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
MHB2	Bay Point Swansea	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
MHB4		In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
MHB5	Globe Village Fall River	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization
MHB11	Number 10 Fall River Buoy	In-Situ Grab Sample	X	X	X	X	X	X	Wet weather marine water characterization

Appendix A.5. Map of Estuarine Stations in Mt. Hope Bay and Kickemuit River.

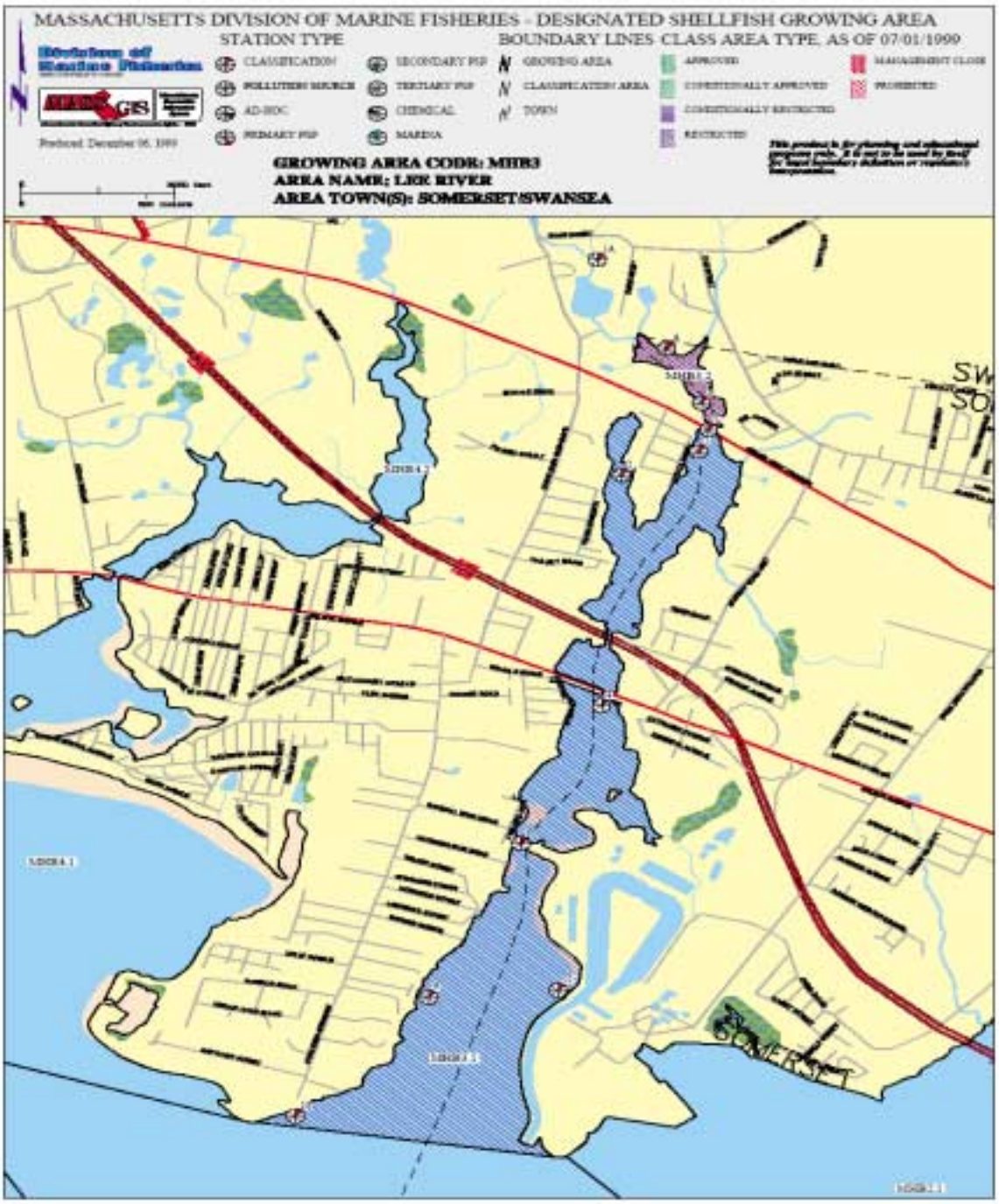
See Figures 5.2 and 5.3.

Appendix A.6. MADMF Sampling Stations in Mount Hope Bay and the Taunton River (MHB1) and Locations of Split Samples collected by MADMF staff (See Appendix A.4).

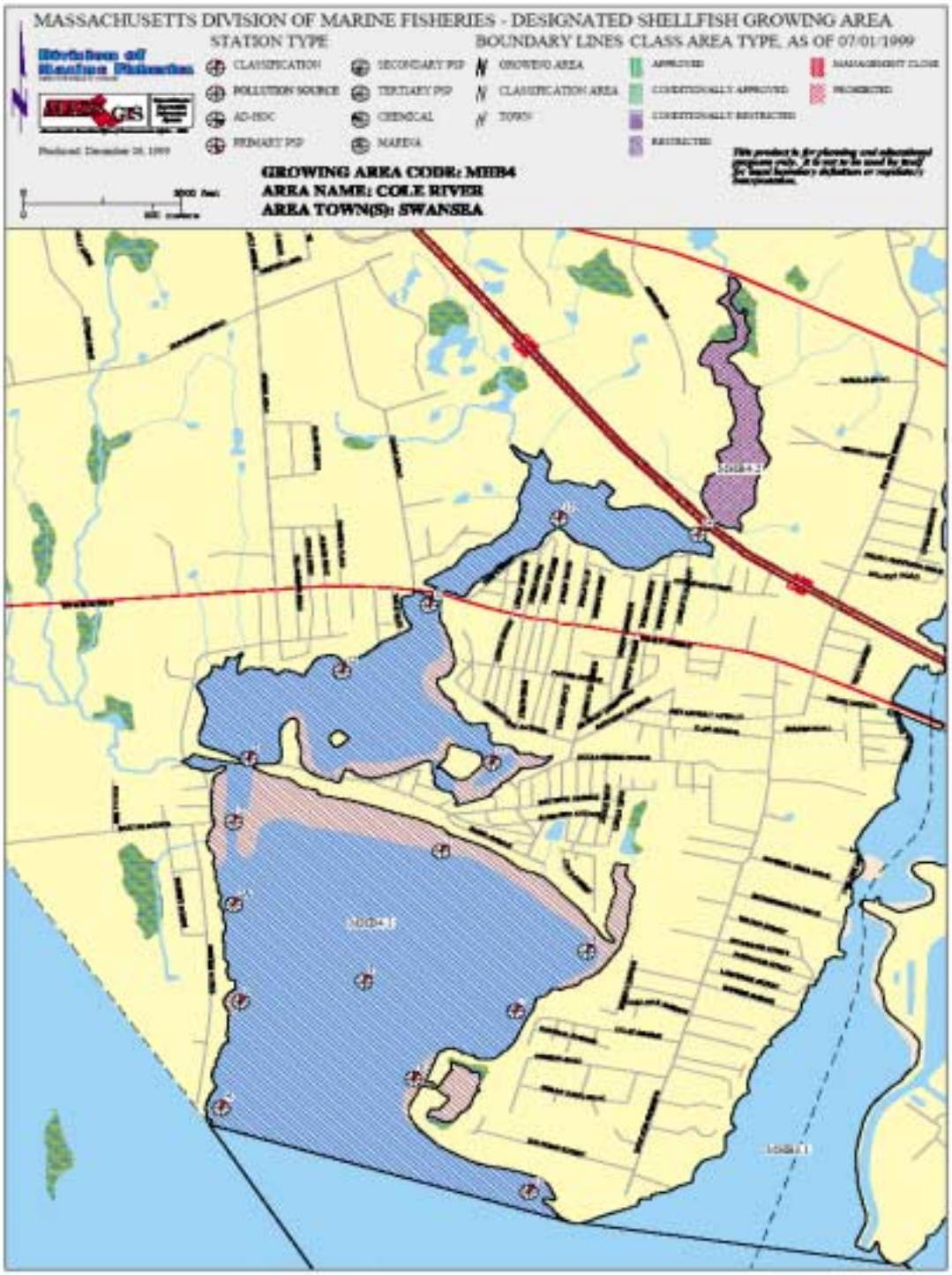


Appendix A.7. MADMF Sampling Stations in Mount Hope Bay and the Taunton River (MHB2).

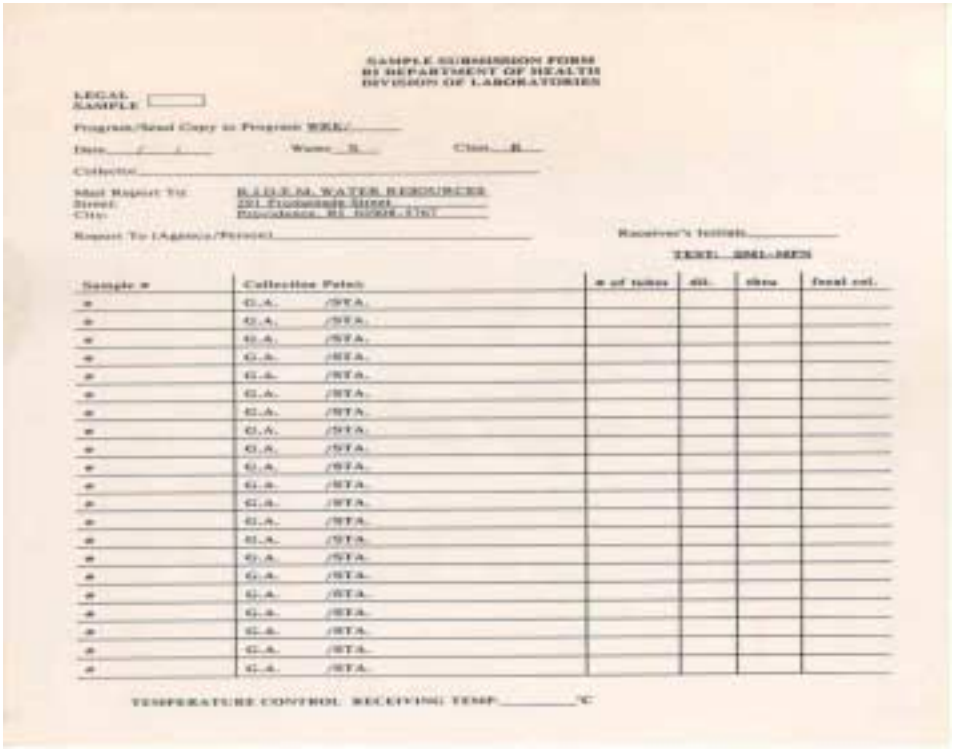
Appendix A.8. MADMF Sampling Stations in Mount Hope Bay and the Taunton River (MHB3).



Appendix A.9. MADMF Sampling Stations in Mount Hope Bay and the Taunton River (MHB4).



Appendix B Chain of Custody Forms



SAMPLE SUBMISSION FORM
RI DEPARTMENT OF HEALTH
DIVISION OF LABORATORIES

LEGAL SAMPLE ☐

Program/Send Copy to Program WRK/_____

Date ___/___/___ Water ___S___ Class ___B___

Collector _____

Mail Report To: RHODE ISLAND WATER RESOURCES
Street: 235 Promenade Street
City: Providence, RI 02908-5767

Report To (Agency/Person) _____ Receiver's Initials _____

TEST: 9041-9879

Sample #	Collection Point	# of tubes	dil.	thru	final vol.
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				
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#	G.A. /STA.				
#	G.A. /STA.				
#	G.A. /STA.				

TEMPERATURE CONTROL RECEIVING TEMP: _____°C

Appendix B.1 Department of Health Chain of Custody Form.

Attachment A. Field Sampling Standard Operating Procedures.

A.1. Sampling SOP 1 (S-1): Fecal Coliform/Male Specific Bacteriophage Sampling

1. The laboratory-provided autoclaved sample bottles will be distributed to each team.

2. The following information is on the sample bottle label. The fields that should be filled in prior to sampling are in bold below. Label the bottle before taking the sample. The Station field should be composed of the Station ID followed by the hour when the sample was taken. A sample taken at hour 4 at Station GA17-2 would become GA17-2-4.

   Sample Bottle Label

Station:		
Depth:	Temp:	**Date:**
Initials	Salin.	**Time:**
Sample Type:		
Remarks:		

   The fields in **bold**, the station, date, time, and initial fields should be labeled on the sample bottle.

3. If you are using a sample stick, place the bottle in the stick.

4. When you are ready to take the sample, take the cap off the sample bottle. Hold the cap in one hand and the bottle (in the sample stick) in your other hand. Do not touch the inside of the bottle or cap. Do not put the cap on the ground.

5. Avoid contaminating the samples by not allowing the sample water to come in contact with anything before it is placed in the bottle. Be careful not to bring the rim or cap of the sample bottle into contact with anything. If possible, samples will be taken with a sample stick to avoid causing upstream disturbance prior to and during sampling.

6. Holding the bottle upside down, push the bottle through the water to mid-depth or as far as you can reach. Turn the bottle forward and scoop it forward and up and out of the water. Do this in one sweeping motion. Make sure you sample forward and away from you so that there is no chance that you will contaminate the sample with bacteria from your arm.

7. Pour off water to the neck of the bottle. Water should reach to within an inch to an inch and half of the top of the sample bottle. This provides space for mixing.

8. Cap the bottle tightly. Place the bottle upright in a cooler with ice to maintain a temperature of 4°C.

9. Be sure to record the time that the sample was taken in the sample log.

10. All bacteria samples (including field duplicates) should be taken using the steps discussed in Step 1 through Step 9.

Attachment B. Fixed Laboratory Methods and Standard Operating Procedures.

B.1. Fixed Laboratory Method 1 (L-1) RIDHL MPN Method for Detection of Fecal Coliform Bacteria.

1. **TITLE: MULTIPLE TUBE FERMENTATION TECHNIQUE FOR THE MEMBERS OF THE TOTAL COLIFORM AND FECAL COLIFORM GROUP**

2. **References:** STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 19th EDITION. Section 9221 A,B&E pp.9-44-9-47, 9-51

3. **Scope and Principle of the Analysis:**
   3.1. The coliform group consists of several genera of bacteria belonging to the family Enterobacteriaceae. The historical definition of this group has been based on the method of detection, which is lactose fermentation, rather than on the tenets of systematic bacteriology. When the fermentation technique is used the coliform group is defined as all facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48h at 35°C. The fecal coliform test is applied to determine which total coliforms are also fecal coliforms
   3.2. When the Multiple Tube Fermentation Technique is used the results of the examination of replicate tubes and dilutions are reported in terms of Most Probable Number of organisms present. This number, based statistical probability formulas, is an estimation of mean bacterial density in the sample. This technique is used to determine the estimated bacterial density of non-potable samples and that the quality of potable water meets U.S E.P.A standards.
   3.3. Water of drinking water quality, non-potable water and brackish or salt water as well as mud, sludge and sediment samples can be analyzed by this technique
   3.4. This Standard Operating Procedure varies slightly depending of the matrix. The following procedure can be applied when testing non-potable water and brackish or salt water as well as mud, sludge and sediment samples.

4. **Interferences:** Method interferences may be caused by contaminants in solvents, reagents, glassware and other sample processing apparatus that lead to discrete artifacts. All reagents and apparatus must be routinely demonstrated to be free from significant interferences under the conditions of the analysis by:
   4.1. Sample bottles are autoclaved prior to sample collection. The bottles are checked for sterility before they are allowed out in the field.
   4.2. Glassware must by scrupulously cleaned and instructions for this cleaning should be described and performed as in Section 6.
   4.3. Only reagent-grade water that meets EPA/FDA criteria is used to prepare media and reagents. See table below for microbiological suitable water specifications.

TEST FOR THE FOLLOWING PARAMETERS	LIMITS	FREQUENCY
CONDUCTIVITY	> 0.5 MEGOHMS (resistivity)OR <2 microsemiens	MONTHLY
METALS: Pb, Cd, Cr, Cu, Ni, Zn	NOT GREATER THAN 0.05 mg/L PER CONTAMINANT	ANNUALLY
TOTAL CHLORINE RESIDUAL	<0.1ppm	MONTHLY
HETEROTROPHIC PLATE COUNT (HPC)	< 500 CFU/ML	MONTHLY
pH	6.0-7.5	AS USED
BACTERIOLOGICAL QUALITY OF RINSE WATER	RATIO 0.8-3.0	ANNUALLY

4.4. Inadequate mixing of the sample will result in an underestimation of the actual bacterial density.
4.5. Analysts need to wash hands thoroughly and wipe down the bench with disinfectant before analysis.
4.6. Valid test results require strict adherence to all Quality control procedures.

5. Safety Issues

5.1. . All media is purchased as a dehydrated powder. Analyst should wear gloves and a mask to avoid breathing the dust when weighing and mixing.
5.2. MSDS's for all media components/reagents used in the laboratory are located on a shelf at the end of aisle 5 in a black three ring binder.

6. Apparatus and Equipment

6.1. Sample Bottles: Sample collection bottles should be wide-mouthed plastic (or non-corrosive glass-used only for mail-ins) and have a minimum capacity of 125mL and are able to withstand repeated autoclaving. Sample collecting containers- (sodium thiosulfate added prior to sterilization if needed), sterilize by autoclave for 30 min at 121°C. Sterility, detergent residue and the effectiveness of sodium thiosulfate are checked for each batch of bottles that are autoclaved, results are recorded.
6.2. Washing of sample bottles is performed in the washroom on the third floor. The washing machine is programmed to deliver a complete wash with suitable detergent for microbiological analysis followed by three fresh water rinses and a final rinse with distilled water. Sample containers returned to the lab from the washroom are checked for residue using 0.04% bromothymol blue (records are maintained for each batch of glassware).

6.3. Instrumentation
- 6.3.1. Incubator capable of holding the temperature at 35.0±0.5°C
- 6.3.2. Water bath capable of holding the temperature at 44.5±0.2°C.
- 6.3.3. pH meter- Accuracy and graduation scale to ±0.1 units.
- 6.3.4. Autoclave– Operation at 121±2°C
- 6.3.5. Hot air oven- Operation at 170±10°C
- 6.3.6. Balance-capable of detecting 0.1g with a150.0g load.

6.4. Other Equipment
- 6.4.1. Inoculating wire loops made of aluminum or stainless steel, size at least 3mm, which have been sterilized by dry heat at 170°C for two hours.

7. Reagents, Solutions:

7.1. REAGENT–GRADE/MICROBIOLOGICAL SUITABLE WATER

7.1.1. Reagent–Generated through a Barnstead/Thermolyne NaNOpure DIamond TOC Life Science (UV/UF with TOC) ULTRAPURE WATER SYSTEM located in the washroom on the third floor. This system provides water that meets the standards put forth by the E.P.A. (Regulations are stated in section 4.4). This water is used for all phosphate buffered water dilution water and media.

7.2. STOCK PHOSPHATE BUFFER SOLUTION

7.2.1. Prepare by dissolving 34.0g potassium dihydrogen phosphate (KH_2PO_4), in 500mL reagent grade water, adjust to pH 7.2±0.5 with sodium hydroxide (NaOH), and dilute to 1L with reagent grade water. Dispense in 100mL volumes, autoclave for 15 min. at 121^0C. Discard any bottle that has precipitate or is cloudy. Check for sterility by Standard Plate Count Method and record results. Label and store in refrigerator. Record in stock phosphate section of Q.C. RECORDS book.

7.3. STOCK MAGNESIUM CHLORIDE

7.3.1. Prepare by dissolving 81.4g $MgCl_2 \cdot 6H_2O$ in reagent- grade water and make up to a 1L volume with reagent-grade water. Dispense in 100mL volumes, autoclave for 15 min. at 121^0C. Discard any bottle that has precipitate or is cloudy. Check for sterility by Standard Plate Count Method and record results. Label and store in refrigerator. Record in magnesium chloride section of Q.C. RECORDS book.

7.4. BUFFERED WATER (*RINSE/DILUTION WATER)*

7.4.1. Prepare by adding 1.25mL stock phosphate buffer solution and 5.0mL magnesium chloride solution per liter of distilled water. Buffered water is dispensed into bottles fitted with nozzles and sterilized by autoclaving for 30 min. Q.C. 20mL from each bottle is added to 100mL of lactose broth and incubated for 48h as a sterility check. Any bottle exhibiting growth is discarded. Record in rinse water section of Q.C. RECORDS book.

7.5. 10% SODIUM THIOSULFATE $Na_2S_2O_3$

7.5.1. To prepare mix 15.8 grams of $Na_2S_2O_3$ with 200mL reagent water. Filter sterilize solution using the membrane filter apparatus. Label and store in refrigerator. This solution is added to sample collection containers in the amount sufficient to provide an approximate concentration of 100mg/L in the sample. This concentration is obtained by adding 0.5mL to a 4oz. bottle or 0.8mL. to the 8oz. prior to sterilization. The reagent acts as a chlorine neutralizer. Medium is made in clean, borosilicate glass, pre-sterilized, screw capped flasks, according to the manufacturer's directions. Q.C. Perform a heterotrophic plate count on the filter-sterilized reagent, there should be <1CFU/plate. If >1CFU/plate is detected, discard reagent and prepare it again. Record in sodium thiosulfate section of Q.C. RECORDS book.

7.6. DISINFECTANT

7.6.1. Amphyll- Prepared in laboratory by measuring 200mL F/H® Disinfectant-Virucide Sanitizer-Cleaner for Hospital and Institutional Use and adding it to 18L of distilled water. This solution is prepared and stored in a carboy.

8. Media:

8.1. LAURYL TRYPTOSE BROTH (LTB)*

8.1.1. Prepared according to manufacturer.

8.1.1.1. The table below shows, grams needed to prepare single strength and double strength Lauryl Tryptose Broth.

Liters	***1X***	***2X***
2	71.2gm	142.4gm
3	106.8gm	213.6gm
4	142.4gm	284.8gm
5	178.0gm	356.0gm

6	213.6gm	427.2gm

8.2. BRILLIANT GREEN LACTOSE BILE BROTH (BGLBB)*

8.2.1. Preparation: according to manufacturer. Record quantity made, lot #, pH, etc. in media logbook.

8.3. EC BROTH*

8.3.1. Preparation: according to manufacturer. Record quantity made, lot #, pH, etc. in media logbook.

8.4. All tube media/broths are dispensed in 10mL amounts into fermentation tubes [size – 16x125 (EC, BGLBB, A-1 and LTB 1X) or 20x150 (A-1 and LTB 2X)] equipped with inverted inserts (unless otherwise directed).

8.4.1. Volume is checked at the start of the filling process and after autoclaving, volume should be 10±2.5%. Perform weekly and record in the Media Volume section of the Q.A. RECORDS book.

8.4.1.1. Using a couple of representative tubes, without a durham tube insert, dispense media as usual. Then measure volume in a 10 ml graduated cylinder (calibrated with a class A annually).

8.5. Media is autoclaved as directed by manufacturer.

8.5.1. Do not allow media to remain in autoclave for more than 30min.

8.6. All media has the pH measured before (if needed) and after autoclaving. If needed, the pH is adjusted (<0.5 units) prior to autoclaving, by adding either sodium hydroxide or hydrochloric acid.

8.7. All media is purchased as a dehydrated powder. Lot #'s are recorded in the new lot #/ media section of Q.A. RECORDS book

8.7.1. Bottles are dated upon receipt and when opened and are labeled with a laboratory expiration date which is 6 months from the opening date.

8.7.2. Powdered media is kept in a cool dry place.

8.7.3. Discard any powdered media if it appears caked, discolored.

8.8. Prepared media in tubes is dated when prepared and /or laboratory expiration date (tubes with loose fitting caps at room temperature are good for 7.days. Tubes with screw caps at room temperature are good for 1 month. Tubes with loose fitting caps refrigerated at 0-4.0C are good for 2 weeks, and tubes with screw caps refrigerated at 0-4.0C are good for 3 months. Prepared media is stored in a cool dry, dark place or in the refrigerator

8.8.1. A couple of representative tubes from every batch prepared are marked with a "sharpie" immediately after autoclaving at the volume height. Media should be discarded if evaporation loss is >1ml at the time of use.

8.8.2. Refrigerated media is allowed to come to room temperature. Remove from refrigerator 24h prior to use.

8.8.3. Prepared media is discarded if any growth or any air bubbles are present.

8.9. LEVINE'S EOSIN METHYLENE BLUE (EMB)

8.9.1. Purchased plate media used to grow positive tube cultures for gram staining confirmation.

9. Sample Collection, Preservation and Storage

9.1. Sample collection bottles are wide-mouthed plastic or non-corrosive glass and a minimum capacity of 125mL that are able to withstand repeated autoclaving.

9.1.1. After clean samples bottles are returned from the washroom and have dried sufficiently they are capped and ready to be sterilized by autoclaving for 30 min at 121^0C. Sterility and the effectiveness of sodium thiosulfate, if added, are checked for each batch. Pouring 50mL of a non-selective medium (tryptic soy broth or Lauryl tryptose (1X) broth) into the bottle

followed by incubation for 24-48H and observed for growth checks sterility of sample bottles. Results are recorded in the sample bottle section of Q.C. RECORDS book. The effectiveness of sodium thiosulfate is conducted by adding a solution of bleach and water (1ml bleach/500mL water) to the bottle then adding a drop of Orthotoludine. This reaction should not produce a color change if the sodium thiosulfate is working correctly.

9.1.2. Sodium thiosulfate (pipette 0.5mL to a 4oz. bottle or 0.8mL. to the 8oz.) is added prior to sterilization. Samples containers with sodium thiosulfate added are used for chlorinated test sites.

9.1.3. Sample bottles are then labeled and stamped with the date of sterilization.

9.1.4. Samples are collected, then held at 0-10.0ºC during a maximum transport time of 6 hours.

9.1.5. Once at the laboratory samples are held under refrigeration until analysis (within **2 hours** of receipt).

9.1.6. The requirements stated above can be unrealistic, so if needed analysis can be performed on samples when less than 24 hours have elapsed from collection to receipt in the laboratory. An observation of the collection time and date located on the Form A is a prerequisite to acceptance of the sample by the staff in Central Receiving. Laboratory personnel review the collection date and time when samples arrive in the laboratory for analysis.

9.2. There is a 24-hour maximum hold time on water samples. An observation of the collection time and date located on the Form A is a prerequisite to acceptance of the sample by the staff in Central Receiving. Laboratory personnel review the collection date and time when samples arrive in the laboratory for analysis.

10. Quality Control:

10.1. Known samples, accompany samples throughout incubation processes to insure that the proper temperatures are maintained. Daily quality control for media: Inoculate 4 tubes each of BGLBB, EC, and LTB 1X and 2X with *E.coli, Enterobacter aerogenes and H.alveii*, last tube is uninoculated for sterility. Incubate BGLBB, 1X and 2X tubes at $35^{+}_{-}0.5$°C for 24h., and EC tubes at 44.5±0.2°C for 24 h.

10.2. Results are recorded every day and should be;

10.2.1. BGLBB, 1X, 2X - growth and gas for *E.coli* and *Ent.aerog* growth and no gas for *H.alveii* no growth or gas in uninoculated tube.

10.2.2. EC - growth and gas for *E.coli* only - no gas or growth for *Ent.aerog.*, *H.alveii*, or uninoculated tubes.

10.2.3. All new media of LTB, EC, BGLBB are checked as above when made.

10.3. Sterility of pipettes is checked by pippeting sterile water into a tube containing LTB for each new lot#.

10.3.1. Records are maintained in Purchased equipment sterilized by manufacturer section of Q.C. Book

11. Sample Preparation:

11.1. Analysis performed within 24h of collection.

11.2. Keep under refrigeration until time of analysis (within 2h of receipt).

12. Procedure:

12.1. **Presumptive Phase**

12.1.1. Work areas are wiped down with germicide prior to testing.

12.1.2. All tubes are checked for the presence of gas prior to inoculation, if any are found they are discarded.

12.1.3. Appropriate amounts of sample are determined. If sample is drinking water the standard volume is 10mL in 10 tubes. For other waters use multiples and sub-multiples of 10 to inoculate tubes. Volume of sample and medium should result in single strength medium

12.1.4. Tubes containing lauryl tryptose broth (LTB), 1X and 2X, broth are set up in racks, such that there are 12 tubes per sample.

12.1.4.1. **EXAMPLE:** Standard 3 tube, $10\text{-}10^{-2}$ dilution

12.1.4.1.1. First Row - Start with 2x(LTB), arrange in rack 3 tubes deep. Add 10 ml of sample in each of the three tubes.

12.1.4.1.2. Second Row- 1x(LTB), arrange in rack 3 tubes deep. Add 1ml of sample in each of the three tubes.

12.1.4.1.3. Third row-2x(LTB), arrange in rack 3 tubes deep. Add 10 ml of a 1:100 dilution of original sample in each of the three tubes.

12.1.4.1.4. Fourth Row-1x(LTB), arrange in rack 3 tubes deep. Add 1 ml of a 1:100 dilution of original sample in each of the three tubes

12.1.4.1.5. A 1:100 dilution is made by adding 1ml of original sample to 99ml dilution blank (phosphate buffered water).

12.1.4.2. Wastewater is usually analyzed using a 3 tube, $10\text{-}10^{-5}$ dilution.

12.1.4.2.1. This setup is accomplished by setting up rack as above with a fifth, sixth, and seventh row added.

12.1.4.2.2. Inoculate the same as above, making two more decimal dilutions (1ml into 99ml dilution blank (phosphate buffered water).

12.1.5. Samples and dilutions are shaken vigorously (25 times through a 1ft arc/7s) before any sample is withdrawn.

12.1.6. Dispense appropriate volumes of sample and dilutions using sterile pipettes and a pipetting aid (mouth pipetting is not permitted) into Lauryl Tryptose Broth (1x and 2x) as a presumptive test.

12.1.7. Tubes are then incubated 35±0.5°C. After 24h±2h tubes are gently swirled and observed for growth with or without gas (bubbles in the inverted insert). Record presence or absence of growth, gas, and acid production. Negative tubes are reincubated for another 24h. All positive and negative tubes are recorded. All presumptive positive tubes after 24 h or 48h are subjected to the confirmation and completed phase.

12.2. **Confirmation Phase and Fecal Coliform Test**

12.2.1. Brilliant Green Lactose Bile Broth is used for the confirmed test.

12.2.1.1. This test is performed not performed for shellfish harvesting areas.

12.2.2. EC medium is used for the fecal coliform test.

12.2.3. Using a sterile loop 3.0-3.5mm in diameter, transfer one or more loopfuls from each positive presumptive tube and inoculate a corresponding BGLBB tube and EC tube.

12.2.3.1. Incubate BGLBB tubes at 35±0.5°C. Tubes are checked at 24±2h and 48±3h for gas production.

12.2.3.1.1. Any amount of gas formation in the inverted vile after 48±h constitutes a positive test

12.2.3.2. Incubate EC tubes in water bath at 44.5±0.2°C. Tubes are read at 24±2h for gas production.

12.2.3.2.1. EC must be incubated within 20min. of inoculation.

12.2.3.2.2. Water level in bath must immerse upper level of medium.

12.2.3.2.3. Gas production with growth within 24±2h constitutes a positive fecal coliform reaction.

12.2.4. All positive and negative tubes are recorded.

12.3. **Completed Phase**

12.3.1. At least 10% of positive confirmed tubes are subject to the completed test phase. Positive BGLB tube is streaked onto Levine EMB agar plates for colony isolation and incubated at 35±0.5°C for 24h(Coliform morphology-Typical- is pink-dark red with a metallic green surface, Atypical- is pink, red, white, or colorless without a green sheen). Growth from coliform colonies is transferred to LTB medium and a nutrient agar slant (the latter can be omitted for drinking water samples). LTB tubes are incubated at 35±0.5°C for 24h-48h. If gas produced in LTB then cultures are considered coliforms. Optional for drinking water samples, agar slant is incubated for 18h-24h, then gram stain a portion of agar slant. Observe microscopically for coliforms (gram-negative rods, no spores present).

13. Laboratory Action:

13.1. The sequence of positive and negative tubes for the presumptive, confirmed, fecal tests and a calculated MPN value are entered into the Aspen system. The system electronically sends results to the appropriate offices.

14. Expression of Results:

14.1. Final results are based on the confirmatory test for total coliforms and fecal coliforms.

14.2. If multiple tubes are used, an MPN can be calculated. If only one tube is used for subculturing from a single presumptive bottle, report as present or absent for total and fecal coliforms.

14.3. Coliform density values can be found on the MPN index and 95% Confidence Limits Tables.

14.3.1. There are a variety of these tables available depending on the planting series used.

15. Reporting

15.1. Prior to entering results electronically, worksheets must be reviewed.

15.1.1. Daily data worksheets are accepted or rejected on the basis of correctness and completeness by a laboratorian. Date and initials are required.

15.1.1.1. Any worksheets that are rejected are brought to the supervisor's attention for a corrective plan of action.

15.1.1.2. Acceptable work sheets are ready to be entered into the Aspen system.

15.2. Entering results into the Aspen system.

15.2.1. An adhesive label designates each sample with a six-digit number that is applied to bottle in the field or upon receipt in the Central Receiving area.

15.2.2. Electronic entering of data requires a new number be assigned called an Aspen #.

15.2.2.1. This is achieved in Central Receiving where specimens are initially entered into Aspen.

15.3. Retrieving the Aspen number from the Aspen workstation.

15.3.1. Log into the system with the appropriate user designation and password.

15.3.2. Click on the first tab under the Active Sample column-**Log in/Edit.**

15.3.2.1. Scroll through the bottom left corner of the field marked "Record". When you encounter a sample that matches the six digit # found on the adhesive label applied by the collector in the "Lab ID" field record the Sample I.D # (Aspen #) in the appropriate spot the worksheet.

15.3.2.2. Each sample now has two unique numbers assigned to it. (An adhesive label with six digit number that is applied in the field or upon receipt in the Central Receiving office by the collector and the Aspen #).

15.3.3. Assigning a Worklist #;

15.3.3.1. Click on the enter sample results tab (2nd under the Login/Edit column).

15.3.3.2. Click on "Create New Worksheet" tab.

15.3.3.3. Click on "By Test Group" tab.

15.3.3.4. Move cursor to the "Select Test Group ID" field.

15.3.3.5. Using the drop down menu scroll to the analysis test code (SM01).

15.3.3.6. Hit "Filter Test Group".

15.3.3.7. All samples that are pending with the test code of the previous step will be listed.

15.3.3.8. Your cursor will automatically be in the "Select For Data Entry" field. Click on each sample that you want to group on one work list.

15.3.3.8.1. Daily work lists should contain all samples for each day for each test code.

15.3.3.9. Hit "Enter Results".

15.3.3.9.1. A new screen will appear showing the Worksheet #. This number is recorded in the workbook.

15.4. Entering results;

15.4.1.1. Click on the enter sample results tab (2nd under the Login/Edit column).

15.4.1.2. Click on "Review Existing Worksheet" tab.

15.4.1.2.1. Scroll to the worksheet number you want, highlight it and press continue.

15.4.2. All results are entered in the "Alpha Results" field.

15.4.2.1.1. It is necessary to hit enter several times after the last result is entered.

15.4.2.2. Next hit the "Mark Status" tab.

15.4.2.3. Select the "Mark Tests Analyzed". Hit "O.K".

15.4.2.4. Type in date of analysis, when prompted.

15.4.2.5. Type in initials of analyst (person who actually performed test), when prompted.

15.4.2.6. Always use the "Back" button to get out of an Aspen screen. When the first screen is reached, hit the "Stop" button to shut down Aspen.

15.4.2.7. The workbook is initialed and dated in the space marked Entered.

15.5. Reviewing Aspen entry;

15.5.1. A person other than the person who did the original entering reviews results in a timely fashion.

15.5.2. To review, hit the "Routine Reports" button in the Login/Edit column on Aspens first screen.

15.5.3. Select "Worksheets".

15.5.4. Select the work list # you want to review.

15.5.5. Review entered results and accepted workbook results for accuracy.

15.5.6. Attention should be made to assigned numbers to see that they correlate.

15.5.7. If a mistake is encountered print the page containing the mistake.

15.6. Changing an incorrect result.

15.6.1. In the first screen of Aspen hit enter results button.

15.6.2. Select the appropriate work list.

15.6.3. Scroll to the field where the mistake is found.

15.6.4. Make the needed changes and hit enter twice.

15.6.5. Print page with corrected results.

15.6.6. Fill out a corrective action report form and affix the incorrect and the corrected Aspen pages.

15.7. Once the Aspen results are reviewed and corrected if needed the worklist can be marked ready to report.

15.7.1. Begin by following section 15.4 – 15.4.2.2.

15.7.2. After hitting the "mark status" button, highlight the " mark tests approved" bullet.

15.7.3. Type in date approved (today's date), when prompted.

15.7.3.1. Type in initials of person who approved worklist (person reviewing results on Aspen), when prompted.

15.7.4. The workbook is initialed and dated in the space marked Release/Approve.

15.8. Always use the "Back" button to get out of an Aspen screen. When the first screen is reached, hit the "Stop" button to shut down Aspen.

B.2. Standard Operating Procedures (SOP) for determination of male-specific bacteriophage.

Procedure for Determining Male-specific Bacteriophage In Seawater and Wastewater

The protocol is based on the work of Dr. William Burkhardt, US FDA, Dauphin Island, Alabama. He taught the procedure to the Northeast Laboratory Evaluation Officers and Managers (NELEOMs) at the Male-specific Bacteriophage (MSB) Workshop in Gloucester, Massachusetts, on March 9-12, 2004.

Coliphage are bacterial viruses (bacteriophages) that infect and replicate in *Escherichia coli*. They are often found in high concentrations in municipal wastewater and to a lesser degree in human and animal feces. Coliphages are potentially important microorganisms for monitoring the microbial quality of waters because traditional bacterial monitoring does not accurately indicate the presence of non-bacterial organisms such as human pathogenic viruses; human virus detection is beyond the capabilities of most water laboratories; and coliphage detection is relatively inexpensive, easy to perform, and provides overnight results.

The male-specific coliphages infect male bacterial cells via the F-pilus. F-factor is the fertility factor in certain strains of *E. coli*. It is a plasmid that, when present, codes for pilus formation. The pilus allows the transfer of nucleic acid from one bacterium to another. Male-specific coliphages (F+) are RNA or DNA viruses and are unable to replicate in the environment. Although coliphages are not pathogenic for humans, other microorganisms that are human pathogens may be present in waters impacted by untreated or inadequately treated domestic wastes. Use good laboratory practices when working with potentially harmful samples.

The host bacteria used is *E. coli* F_{amp} for male-specific coliphage.

What are male-specific bacteriophages—

$ Lytic viruses of bacteria – (killing of host bacteria).
E. coli and *S. typhimurium*
(*production of E. coli* pili)

$ Requires a piliated host cell for adsorption, they do not attach to cell surface (somatic).

$ Requires host cell in log-phase of growth-cells do not produce pili at < 30°C.

$ Optimal growth temperature: 35 - 37°C.

$ Plaque size is generally self-limiting.

Norwalk-like viruses are presently referred to as Noroviruses. Gastroenteritis symptoms are indicative of viral infection. Norovirus infection causes vomiting, diarrhea, cramps and weakness within an 18-30 h onset with an illnesses lasting for up to 72 h. In addition, Noroviruses are highly contagious and spreads in a confined population (i.e., a cruise ship). Viruses like cold reduced sunshine environments. Bioaccumulation within shellfish in the U.S. occurs from October through January.

Fecal coliforms have no correlation to the bacteriophage levels during outbreak periods normally observed from November through January.

Apparatus and Materials

Equipment for collection and transport of samples—
Sterile bottles with screw caps, 100 mL or 125 mL treated w/ sodium thiosulfate and untreated
4 mil plastic bags
Ice chest
Ice
Latex gloves
Temperature Control Blank
Disinfectant

Equipment for the laboratory—
Centrifuge, sterile 50 mL conical tubes, 9000 x *g* performance capability, 4°C
Water Bath, 44-52°C
Laboratory Balance, 0.00 g
Incubator, 35-37°C
Balance
Stir Plate
Rotator
Vortex
Blender
Autoclave, 121°C
Cold Room, 4°C
Freezer, -20°C
Light Box (optional)
Thermometers, range 0 – 121°C
Timer

Erlenmeyer Flasks, 1 L and 2 L
Graduate Cylinders, 1000 and 500 mL
Stir Bar
Funnel
Shovel
Weigh Boats
Deionized or Distilled Water
Inoculating Loops (3 mm in diameter or 10 ΦL volume)
Sterilization Filtration Equipment - 0.22 or 0.45-µm pore size
SyringesBsterile disposable, 5-, 10- or 20 mL
Potable Water Source
Scrub Brushes, sterile
Bone Snips, sterile
Knives, sterile
Blender Jars, sterile
Baskets, drying
KimWipes
Decontamination Bag

Disinfectant

Pipets-Pipettors, 500 µL, 1000 µL, 5 mL, 10 mL and 25 mL
Pipet Bulb
Centrifuge Tubes, sterile disposable 15 & 50 mL
Petri Dishes, sterile disposable 100 x 15 mm
Test Tubes 16 x 150 mm, s/s loose fitting caps or screw caps
Test Tube Rack--size to accommodate tubes
Freezer Vials- sterile 5 mL screw cap
Foil
Parafilm
Permanent Marker

Reagents
Reagent Water
Glycerol- sterile
Ethanol - 70% or laboratory disinfectant
Calcium Chloride- 1M

Antibiotic stocks
*****Antibiotics must always be added to medium after the medium has been autoclave and cooled.
Streptomycin/Ampicillin (50 µg/mL)
ampicillin sodium salt (Sigma A9518)
streptomycin sulfate (Sigma S6501)

MediaB
Bottom Agar
DS Soft Agar
Growth Broth

StockB Bacterial Host
E.coli F_{amp} . *E. coli* HS(pFamp)RR
- originated by Victor Cabelli, University of Rhode Island, Kingston, RI, USA, frozen stock ATCC #700609.

Bacteriophage-
MS2, ATCC # 15597

Media Composition

***E. coli* F_{amp}**

Bottom Agar

Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g
Agar	15.0 g

DI Water	990 ml

With gentle mixing, add all the components to 990 mL of dH_2O in a 2000 mL flask. Dissolve and sterilize.
Sterilize at 121ºC ± 2ºC for 15 minutes.
Allow the agar to equilibrate in the water bath set at 44 - 46ºC.
Aseptically, add 10 mL of Streptomycin sulfate/Ampicillin solution to the flask. (50 µg/mL final).
Temper to 50ºC in the water bath.
Aseptically, pipet 15 mL aliquots into sterile 100 x 15 mm petri dishes and allow the agar to harden.
Store the dishes inverted at 4ºC overnight and warm to room temperature for 1 h before use.
Plates are good for one month at 4ºC.

Streptomycin sulfate/Ampicillin Solution - Dissolve 0.5g of streptomycin sulfate and 0.5g of ampicillin in 100 mL of dH_2O. Filter with the 0.22 µm filter. Store in 10 mL aliquots in sterile containers at 4ºC. Allow to come to room temperature.

DS Soft Agar

Tryptone	10.0 g
Dextrose	1.0 g
NaCl	5.0 g
1M $CaCl_2$	0.5 mL
Agar	7.0 g
DI Water	500 ml

[*$CaCl_2$ Anhydrous FW 111.0, Dihydrate FW 147]

With gentle mixing, add all the components to 500 mL of dH_2O in a 1000 mL flask.
Bring flask to a boil.
Dispense in 2.5 mL aliquots into 16 x 100 mL tubes and freeze (-20ºC) for up to one month.
Place a foil top on the tubes and sealed them in a zip-lock bag.
Sterilize prior to use at 121ºC ± 2ºC for 15 minutes; temper to 50 - 52ºC for no longer than 2 h.

1M $CaCl_2$ - Add 1.11g of $CaCl_2$ to 10 mL on dH_2O. Sterilize by autoclaving at 121ºC for 15 minutes. Tubes are good for one month at 4ºC. Use at room temperature.
[Divalent cations such as Ca+ are used to maintain phage stability and to facilitate efficient phage absorption to host.]

Growth Broth

Tryptone	10.0 g
Dextrose	1.0 g

NaCl	5.0 g
DI Water	1000 ml

With gentle mixing, add all the components to 1000 mL of dH_2O in a 2000 mL flask. Dissolve and dispense into 100 mL NALGENE containers. Fill the containers to 75 mL.
Sterilize at 121ºC ± 2ºC for 15 minutes.
Bottles are good for one month at 4ºC.

Propagation of *E. coli* Famp

Bottom Agar streak plate - Transfer preferable < 1 week old.

10 mL of Growth Broth medium tempered to 35 - 37ºC - **vortex to aerate prior to inoculation.**

Using a sterile 10 µL loop collect material from several colonies and transfer to the broth medium in a sterile 15 mL conical tube.

Gently shake to mix, and incubate at 35 - 37°C for 4 - 6 hours (turbidity $.10^7$cells/mL; O.D @ 540 nm = 0.4). Once turbidity is observed, you can start using the culture (log-phased growth).

Control Plates

For a *negative* control plate add 2.5 mL of Growth Broth to the 2.5 mL DS Soft Agar tube (adding 0.2 mL of *E. coli* F_{amp} is optional, and recommended).

For a *positive* control plate add 0.2 mL of a 4 h culture of host *E. coli* F_{amp} bacterium (approximately 30-80 PFU/mL). Make serial dilutions of your MS2 coliphage culture using 9 and 9.9 mL Growth Broth blanks. The tube should contain 2.5 mL of DS Soft Agar + 0.2 mL of host + 1 mL of MS2 dilution + 1.5 mL of Growth Broth.

Remove the tube from the water bath. Gently mix the tubes in your palm. Pour each tube onto a Bottom Agar plate containing Streptomycin/Ampicillin (50 µg/ml final). Invert and place in an incubator at 35 - 37°C for 18 - 24 h. Examine for plaques and record the results.

MSB Density Determinations in Low Contaminated Water

Concentration Technique

Used for **shellfish Growing Area water** samples. For each water sample prepare (2) Bottom Agar plates and (2) 2.5 Soft Agar tubes for each plate if processing only a 50 mL portion. If processing the entire 100 mL portion prepare (4) Bottom Agar plates and (4) 2.5 Soft Agar tubes. Use a 4 to 6 h *E. coli* F_{amp}.

Always run a positive control because during the summer months you may not find viruses. Also, run a negative control plate where plaque is not seen. Add (2) Bottom Agar plates and (2) DS Soft Agar tubes for the controls.

1. Weigh 100 mL of sea water in a sterile container. [You may need to put **45 mL** each into two centrifuge bottle.]

2. Allow water to warm to RT (20 - 30 min).

3. Add 1 g tryptone and 1 g beef extract to the 100 mL water sample, shake to dissolve. Bubbles will appear in the bottle. [Use 0.5 g of tryptone and beef extract if using 45 mL portions in the 50 mL centrifuge bottles.]

4. Add 10 mL of *E. coli* F_{amp} culture to 100 mL portion or 5 mL to each 45 mL portion - Do not shake. Clean any overflow on the centrifuge bottle with alcohol.

5. Incubate at 35 - 37ºC for 50 min - rotate at 100 rpm. With the shaker inside the incubator, lay the bottles down on the shaker platform and set the speed to a *very gentle* setting.

6. Centrifuged for 15 min. @ 9,000 x *g*; 4 C.

7. Aspirate or decant supernatant - Do Not Disturb Pellet! Decant into a waste container for biohazard sterilization.

8. Use 5 mL of Growth Broth to re-suspend pellet. Run the tryptone up and down over the pellet or vortexing may be needed to breakup the pellet. You do not want to see any clumps!

9. **Split the 5 mL** Growth Broth into two DS Soft agar tubes combine 2.5 mL aliquot of supernatant and 2.5 mL DS Soft agar (tempered to 52ºC). Gently mix contents by rolling the tube between your palms.

10. Each DS Soft agar tube is overlaid onto one Bottom Agar plate containing Streptomycin/Ampicillin (50 µg/ml final). Drag the mixture into a clear area and don=t over swirl the plates.

11. Plates are inverted and incubated for 18 - 24 h @ 35 - 37ºC. After incubation, count the circular zones of clearing (typically 1 to 10 mm in diameter) in the lawn of host bacteria in double agar plates. Report results in PFU/100 mL.

Calculations for Shellfish Growing Area Waters:

< If only a **50 mL portion** is used you must *multiply the sum of your results by 2* to account for 100 mL sample.

(Plate 1 + Plate 2) x 2 = PFU/100 mL

50 mL portion of Water - 12, 14 ≡ 12 + 14 = 26 x 2 = 52 PFU/100 mL

< If the entire **100 mL portion** is used the result is *the sum of all four plates*.

(Plate 1 + Plate 2 + Plate 3 + Plate 4) = PFU/100 mL

50 mL portion of Water - 6, 10 ≡ 6 + 10 + 8 + 7 = 31 PFU/100 mL
50 mL portion of Water - 8, 7

MSB Density Determinations in Highly Contaminated Water

∃ 100 PFU/100 mL

FDA recommends the following—

For a **raw sewage** sample process 2.5 mL from (10^{-1}) and (10^{-2}) dilution. Pour 4 plates for each dilution. Run 2 control plates.

For a **treated sewage** sample process 2.5 mL for the "direct" technique, 2.5 mL from (10^{-1}) and 2.5 mL (10^{-2}) dilutions from the "concentration" technique. Pour 4 plates for each dilution. Run 2 control plates.

Tryptone Dilution Tubes—
Aseptically, dispense 9 mL aliquots of sterile Growth Broth into sterile 16 x 150 mm test tubes that have been sterilized at 121EC for 15 min.

To carry out a serial "ten-fold" dilution you would do the following:

From a 100 mL sewage water sample –

Take 1 mL of the water sample and mix it with 9 mL of Growth Broth. The dilution becomes (1:10). Mix or vortex. Use a micropipette with new sterile tip to carry out a second ten-fold dilution.

Take 1 mL of the (1:10) tube and mix it with a new 9 mL tube of Growth Broth. The dilution becomes (1:100). Repeat until you have reached your desired concentration.

1. Allow the water sample to warm to RT (20 - 30 min).

2. Prepare your serial dilution with the water sample.

3. *When running up to 3 plates per dilution*B Combine **2.5 mL aliquot of sample**, 0.2 ml of *E.coli* F_{amp} to 2.5 ml DS Soft agar (tempered to 52EC). Gently mix contents by rolling the tube between your palms. [Uses 7.5 mL of each dilution tube.]

   *When running up to 4 plates per dilution*B Combine **1.5 mL aliquot of sample**, 1.0 mL of Growth Broth, 0.2 ml of *E.coli* F_{amp} to 2.5 ml DS Soft agar (tempered to 52EC). Gently mix contents by rolling the tube between your palms. [Uses 6.0 mL of each dilution tube.]

4. Overlay onto a Bottom Agar plate containing Streptomycin/Ampicillin (50µg/ml final). Drag the mixture into a clear area and don=t over swirl the plates.

5. Plates are inverted and incubated for 18 - 24 h @ 35- 37ºC. After incubation, count the circular zones of clearing (typically 1 to 10 mm in diameter) in the lawn of host bacteria in double agar plates. Report results in PFU/100 mL

Computations for Sewage samples using four plates per dilution:

$$\textbf{MSB/100 mL} = \frac{\textbf{Avg of plate counts}}{\textbf{mL analyzed/plate}} \times \textbf{dilution factor} \times \frac{100}{100} = \textbf{PFU/100 mL}$$

10^{0} = TNTC, TNTC, TNTC, TNTC
10^{-1} = 164, 190, 170, 175 = Average 174.75 pfu/plate
10^{-2} = 16, 16

$$174.75 \text{) } 1.5 \text{ mL aliquot} = 116.5 \times \frac{1000}{100}* = 116{,}500 \equiv 120{,}000 \text{ PFU/100 mL}$$

[* 1000 ≡ 100 mL (water sample) x 10 (from 10^{-1})]

Storage of *E. coli* F_{amp}

$ No Selective Pressure - Addition of glycerol (10% final) into a broth culture.*
Storage: Freeze at - 80 C Indefinite. (*Most desirable method.)*
[Pressure is reapplied when the culture is streaked back onto the Bottom Agar containing the two antibiotics.]

$ Bottom Agar Streak Plate
Storage: Refrigerator at 4 C for 2-3 weeks.

$ Tryptic Soy Agar Slant w/ Mineral Oil overlay
Storage: Room temperature in the dark for 2-5 years +.

$ Selective Pressure - Tryptic soy Agar Slant w/Streptomycin and Ampicillin. Stab deep, let grow for 6 h, and then overlay with sterile mineral oil. (*Least desirable method.*)

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Glycerol Solution, 10% - Add 9 mL of distilled water to 1 mL of undiluted glycerol. Autoclave resulting 10% glycerol solution at 121EC for 15 min and use at room temperature.

* Add 1/5th volume of 10% glycerol solution. Let stand for 30 min. Dispense into 1 mL aliquots in 2-mL cryovials and store at -70 to -80EC is best, but -20EC is acceptable.

Source of Bacterial Host Strains

E. coli HS(pFamp)R, ATCC #700891- Bacterial host for male-specific coliphages.

Sample Collection and Storage—

< Sterile sample containers of plastic or borosilicate glass are used to collect water samples. 4 mil plastic bags are used to collect shellfish shellstock samples. If the water sample is from a shellfish growing area follow your water and shellfish sample collection SOPs.

< If the sample is from a sewage treatment plate, gloves should be used when the sample is collect. If the sample was chlorinated use a sodium thiosulfate treated sample container. If the sample was unchlorinated use an untreated sample bottle.

< Record all pertinent information of the collection form.

< Maximum holding times for samples-
- raw and treated sewage samples up to 24 h,
- shellfish up to 24 h, and
- shellfish growing area water up to 30 h.

Quality Assurance—

< Run positive and negative control plates with each sample.

< Check media sterility.

< Media log book should be maintained (volume, weights of each components, lot numbers, etc.).

< Develop an intra- and inter-laboratory performance program.

< Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria after 18- 24 h of incubation are plaques. Count the number of plaques on each plate. (Use of a light box to evaluate results is recommended.) Plaque forming unit (PFU).

< Desired range of zero to 300 PFU per plate. If the count exceeds the upper range or if the plaques are not discrete, results should be recorded as *too numerous to count* (TNTC).

Calibration and Standardization—

< Check temperatures in the water baths, refrigerators, freezers, and incubator twice a day (at least 4 h apart) to ensure operation within the stated limits of the method and record the measurements in a log book.

< Check thermometers at least annually against an NIST-certified thermometer.

< Calibrate the balance annually using ASTM-certified Class 1 or 2 or NIST Class S reference weights.

< Laboratories must adhere to all applicable quality control requirements set forth in the most recent version of FDA=s *Shellfish Laboratory Evaluation Checklist*.

Safety—

< Samples, reference materials, and equipment known or suspected to have bacteriophage attached or contained must be sterilized prior to disposal.

Useful Terms—

h - hour
°C - degrees Celsius
µL - microliter
g - gram
L - liter
M - molar
mL - milliliter
rpm - revolutions per minute
x - Atimes@
Ave. - average
Na2S2O3 - Sodium thiosulfate
MSB - male-specific bacteriophage
NIST - National Institute of Standards and Technology
PFU - number of plaque forming units
RT - room temperature
TNTC - too numerous to count

Coliphage - Viruses that infect *E. coli*.

Enrichment - Enrichment is meant as the increase in number of bacteriophage through the addition to the growth medium of host bacteria allowing coliphage replication.

Host Bacteria - Are those bacteria that allow the bacteriophage to penetrate and replicate with them, ultimately lysing, resulting in the release of the progeny bacteriophage. Host bacteria are essential for virus replication. The host used in this method is *E.coli* F_{amp} (*E.coli*HS(pFamp)R).

Lysis zone - In this method, typically a circular zone of clearing indication a sample is positive for coliphages.

Male-specific coliphage - Viruses (bacteriophages) that infect coliform bacteria only via the F-pilus.

Plaque - Circular zones of clearing (typically 1 to 10 mm in diameter) in lawn of host bacteria in double agar plates after incubation.

References:

Enumeration of Male-specific Bacteriophage in Water and Shellfish tissue, William Burkhardt III, Ph.D., U.S. Food and Drug Administration, Gulf Coast Seafood Laboratory, Dauphin Island, Alabama. (Workshop presentation on March 10, 2004.)

USEPA Manual of Methods for Virology, EPA 600/4-84/013 (N16), Chapter 16, June 2001.

Method 1601: Male-specific (F+) and Somatic Coliphage in Water by Two-step Enrichment Procedure, USEPA, EPA 821-R-01-030, April 2001.